THE JOURNAL OF COMPARATIVE NEUROLOGY 466:278–298 (2003)

Collateralization of Climbing and Mossy Fibers Projecting to the Nodulus and Flocculus of the Rat Cerebellum

TOM J.H. RUIGROK*
Department of Neuroscience, Erasmus Medical Center, 3000 DR Rotterdam, The Netherlands

ABSTRACT

Collateralization of mossy and climbing fibers was investigated using cortical injections of cholera toxin b-subunit in the rat vestibulocerebellum. Injections were characterized by their retrograde labeling within the inferior olive. Collateral labeling was plotted using color-coded density profiles of the whole cerebellar cortex. Injections in the medial part of the nodulus resulted in olivary labeling that was restricted to the rostral part of the dorsal cap. Climbing fiber collaterals were found in medial and lateral nodular zones as well as in the ventral paraflocculus and adjacent flocculus. Injections in the intermediate part of the nodulus resulted in olivary labeling of the β-subnucleus but could also involve the ventrolateral outgrowth. In the latter case, climbing fiber collaterals were found in the two floccular zones and in a small region in the lateral-most part of crus I. All nodular injections showed a bilaterally symmetric distribution of collateral mossy fiber rosettes that was mostly confined to the vestibulocerebellum and originated predominantly from the vestibular nuclei. Injections in the flocculus labeled the caudal part of the dorsal cap and/or the ventrolateral outgrowth. Mossy fiber rosettes were observed throughout the vestibulocerebellum but also included other regions of the cerebellar cortex in a bilaterally symmetric pattern corresponding with a more widespread precerebellar origin. Climbing fibers originating in the rostral dorsal cap, labeled from an injection in the ventral paraflocculus, collateralize to a medial and lateral zone in the nodulus. Climbing fiber collaterals were usually accompanied by subjacent labeling of mossy fiber rosettes. These results demonstrate that some nodular and floccular zones are related and, at least partially, share a common input. J. Comp. Neurol. 466: 278–298, 2003.

Indexing terms: vestibulocerebellum; cerebellar zones; Purkinje cells; axon collaterals; cholera toxin b-subunit

The two main afferent systems to the cerebellum, i.e., the mossy fiber and the climbing fiber systems, have very different morphologic and anatomic characteristics. Mossy fibers originate from a multitude of areas in the brainstem and spinal cord and terminate within the granule cell layer, whereas the climbing fibers are all derived from the inferior olive and terminate within the molecular layer where they innervate the Purkinje cell dendrites (for review, see Ruigrok and Cella, 1995; Voogd, 1995). The organization of the olivocortical projection is further characterized by a strict longitudinal patterning such that the neurons from a particular region in the inferior olive terminate on Purkinje cells that are arranged in a narrow, longitudinally arranged, strip (Groenewegen et al., 1979; Voogd and Glickstein, 1998). Since the output of these strips of Purkinje cells each reach different regions of the cerebellar or vestibular nuclei, which constitute the output centers of the cerebellum, it is generally believed that this modular organization of the olivocorticonuclear projection reflects the topographical basis for the diversity of cerebellar function (Voogd and Bigaré, 1980; van der Steen et al., 1991). Mossy fibers, on the other hand, are characterized by a prominent diverging pattern of terminations. Collaterals of particular fibers not only terminate

Grant sponsor: The Dutch Ministry of Education and Sciences; Grant sponsor: European Commission; Grant number: BIO4-98-0182.

*Correspondence to: Tom J.H. Ruigrok, Department of Neuroscience, Erasmus MC, PO Box 1738, 3000 DR Rotterdam, the Netherlands. E-mail: t.ruigrok@erasmusmc.nl

Received 28 April 2003; Revised 9 June 2003; Accepted 16 June 2003
DOI 10.1002/cne.10889
Published online the week of September 22, 2003 in Wiley InterScience (www.interscience.wiley.com).

at multiple locations within the rostrocaudal plane but also in the mediolateral direction and may even reach the contralateral cerebellum (Ruigrok and Cella, 1995; Voogd et al., 1996b; Wu et al., 1999; Serapide et al., 2001).

However, despite that these basic characteristics are well known, it is still unclear to what extent the input to a functionally homologous cerebellar region, i.e., a region that belongs to a particular cerebellar module can collateralize to other related and/or unrelated cortical areas. Although this issue has been investigated for the climbing fiber pathway by using electrophysiological and anatomic techniques (Ekerot and Larson, 1982; Apps et al., 1991; Apps, 2000), this topic cannot be properly examined with ordinary anterograde techniques, because it usually cannot be determined whether terminals are derived from the same or from different neurons. Retrograde double or multiple labeling techniques also have their limitations due to the enormous amount of possible combinations of injections, especially when small injections are required in order to examine potential differences in zonal collateralization (Payne et al., 1985; Payne, 1987; Apps et al., 1991; Apps, 2000; Apps and Garwicz, 2000). Single fiber studies may provide the required information on collateralization but are very tedious (Wu et al., 1999; Sugihara et al., 2001; Sultan, 2001). Recently, Chen and Aston Jones (1998) showed that cholera toxin b-subunit (CTb) is an excellent tracer to demonstrate the labeling of collaterals of climbing and mossy fibers (Voogd et al., 2003). In the present study, this bidirectional tracer technique is used to determine the distribution and the origin of the collaterals from climbing and mossy fibers that terminate in particular zones of the vestibulocerebellum of the rat.

The vestibulocerebellum is generally considered to consist of the nodulus (lobule X) and ventral part of the uvula (lobule IX-C) and, as its hemispheral counterpart, the flocculus (including the adjacent part of the ventral paraflocculus). The flocculus is involved in adaptation of the vestibulo-ocular reflex (Ito, 1984; De Zeeuw et al., 1998), whereas the nodulus has been implicated in the control of postural reflexes (Errico et al., 1996; Barmack et al., 2002). Vestibulocerebellar Purkinje cells project to the vestibular nuclei and, to some extent, to the ventral regions of the lateral cerebellar nucleus (Bernard, 1987; De Zeeuw et al., 1994; Wylie et al., 1994; Balaban et al., 2000). The main climbing fiber input of the flocculus is derived from the dorsal cap (DC) and the ventrolateral outgrowth (VLO), two subnuclei of the inferior olive that receive optokinetic information (Leonard et al., 1988). The main climbing fiber input of the nodulus is provided by the β-subnucleus of the inferior olive which is known to receive direct projections from the vestibular nuclei (Gerrits et al., 1985; Barmack et al., 1993). Mossy fibers terminating in the flocculus originate from the vestibular nuclei, reticular formation, and the raphe nuclei, whereas the nodulus receives vestibular root fibers and an overwhelming projection from the vestibular nuclei (Voogd, 1995). Still, some of the sources of climbing and mossy fibers project both to the flocculus and the nodulus. This holds for the climbing fibers from the DC and the VLO and for the mossy fibers of the vestibular nuclei and the nucleus prepositus hypoglossi. Branching of these climbing and mossy fibers between the flocculus and the nodulus has been confirmed in rabbit with physiological and double retrograde labeling techniques for a small proportion of their neurons (Epema et al., 1990). However, the precise distribution and extent of collateralization between and within flocculus and nodulus is not clear. Moreover, it is not known to what extent floccular and nodular afferents may reach other cerebellar regions. Hence, in the present study, the CTb technique was used to study the spatial distribution of mossy and climbing fiber collaterals from nodulus to flocculus and vice versa as well as their potential distribution to other regions of the cerebellum. In addition, the correlation between mossy and climbing fiber collateralization was investigated.

Abbreviations

DC	dorsal cap
DMCC	dorsomedial cell column
LRt	lateral reticular nucleus
MAO	medial accessory olive
mlf	medial longitudinal fascicle
MVe	medial vestibular nucleus
Pn	basilar pontine nuclei
PO	principal olive
PrH	nucleus prepositus hypoglossi
RtTg	reticulotegmental nucleus of the pons
UBC	unipolar brush cell
VLO	ventrolateral outgrowth

MATERIALS AND METHODS

A total of 34 male Wistar rats (weight, 200–300 g) that all received a CTb injection (List Biological Laboratories, Campbell, CA) into either the flocculus or nodulus were used for this study. All surgical procedures adhered to NIH guidelines, and permission was obtained from the institute's local committee overseeing animal experiments.

Surgical procedures

The general procedures for injecting CTb and for reaching the flocculus have been described in full in a previous study (Ruigrok et al., 1992) and was basically also used for reaching the nodulus. In brief, animals were anesthetized with an intraperitoneal injection of a cocktail of thiazine-hydrochloride (3 mg/kg) and ketamine (100 mg/kg) and placed in a stereotactic frame according to Paxinos and Watson (1986). Additional doses of ketamine were applied intraperitoneally to maintain anesthesia. The squamosal part of the occipital bone was freed of overlying skin and neck muscles, enabling a dorsalward enlargement of the foramen magnum. Subsequently, the atlanto-occipital membrane and dura were opened, thus visualizing the caudal vermis of the cerebellum.

Glass micropipettes with a tip of 10 to 15 μm, filled with CTb (1% w/v in phosphate buffered saline, pH 7.2, 0.1 M), were attached to a conventional electrophysiological set up. In this way, the neuronal activity picked up by the tip of the pipette could be used to guide the pipette and determine the optimal injection site. The nodulus was reached from a horizontal track running parallel to the rostrocaudal axis of the animal. The pipettes mostly penetrated lobule IX-C of the uvula and characteristic Purkinje cell activity, consisting of simple and complex spikes, was usually picked up at a depth between 1.5 and 2.5 mm, thus corresponding with a nodular folium. The flocculus was reached horizontally at an angle of 50–53 degrees with the rostrocaudal axis, penetrating the midline border

of lobule IX-B and -C, and at a depth of approximately 6.0–7.0 mm. Recording Purkinje cell activity at this particular depth was crucial in establishing that the tip of the pipette was positioned within either the paraflocculus or flocculus. The tracer was injected by applying anodal current pulses to the pipette (4 μA: 7 seconds on, 7 seconds off for a period of 10 minutes). Afterward, the pipette was withdrawn, muscles and skin were sutured, and the animal was allowed to recover. In some animals, a second injection was made with a gold–lectin tracer. This retrograde tracer was used for double retrograde labeling of sources of mossy and climbing fibers. However, the results of these injections fall beyond the scope of the present study and will not be reported upon. The gold–lectin injections did not influence or hamper in any way the visualization of the CTb label.

Histology

After a survival period of 5 to 7 days, all animals were deeply anesthetized with an overdose of barbiturate (sodium pentobarbital: 240 mg/kg) and transcardially perfused with an initial flush of 500 ml of 0.9% saline, followed by 1 liter of a solution containing 4% paraformaldehyde, 0.1% glutaraldehyde, and 4% sucrose. The brain was removed and post-fixed in the perfusate for an additional 3 hours and rinsed and stored overnight in 0.05 M phosphate buffer (pH 7.4: PB) containing 10% sucrose. The brainstem and cerebellum were blocked and embedded in gelatin (10%), which was hardened for 2–3 hours in formaldehyde (4%) containing 30% sucrose and then rinsed and stored overnight in PB with 30% sucrose (at 4°C).

Transverse sections of the gelatin blocks were cut at 40 μm on a freezing microtome and were serially collected in eight glass vials with PB, such that each vial contained a complete one of eight series of sections through the cerebellum and brainstem. Every other vial (four vials total) was used for CTb immunocytochemistry, and the remaining vials served as backup.

Sections were incubated in the vials in goat-raised anti-CTb (1:15,000; List Biological Laboratories) in Tris-buffer containing 0.5 M NaCl and 0.5% Triton X-100 (pH 8.6, TBST) for 48 to 72 hours at 4°C under constant gentle agitation. After thoroughly rinsing them in TBST, they were further incubated for 3 hours in biotinylated donkey anti-goat (1:2,000 in TBST; List Biological Laboratories), rinsed in TBST, and incubated overnight in the avidin-biotin complex (ABC Elite, Vector Laboratories, Burlingame, CA). After final rinses in PB, two of the vials were processed with diaminobenzidine (DAB: 0.025% DAB and 0.005% H_2O_2) for 30 to 45 minutes, whereas the other two vials were processed for 15 to 20 minutes with the same medium but in addition containing 0.01% $CoCl_2$, resulting in a black rather than a brown reaction product. After final rinses in PB, sections from each vial were serially mounted on gelatinized slides, air-dried, lightly counterstained with thionine, defatted, and cover-slipped with Permount.

Analysis

Selected cases were examined and plotted by using an Olympus microscope fitted with a Lucivid miniature monitor and Neurolucida software (MicroBrightField, Inc., Colchester, VT). All cerebellar sections of a single vial, representing a complete one in eight transverse series (interval 320 μm) were examined for mossy and climbing fiber labeling. Contours were plotted by using a 2.5× objective, whereas neuronal profiles, mossy and climbing fiber terminals were examined with a 20× objective and using the automated scan option of the plot program (see Fig. 2). At this magnification, mossy fiber rosettes could usually be clearly distinguished, but in occasional cases of doubt, a 40× objective was used to check the identity of a labeled structure. These plots were also used to count numbers of retrogradely labeled neurons in various precerebellar regions (Table 1). Labeling in the inferior olive was plotted in two alternating vials resulting in a one in four transverse series (interval, 160 μm). Photomicrographs were made with a Leica DMR microscope equipped with a digital camera (Leica DC-300). Corel Photopaint software was used to compensate for brightness and contrast; photo panels were constructed by using CorelDraw. Terminology of olivary subdivisions was adapted from Ruigrok and Voogd (2000).

To visualize the density of labeled mossy fiber terminals over the cerebellar cortical surface, the plotted serial sections were used to construct a flattened and more or less continuous diagram of the cerebellar surface. This procedure basically resembles that used for unfolding and flattening the caudal vermis as used by Voogd and Ruigrok (1997) but continues over all cerebellar foliations. The continuation of the lobules is rather straightforward and clearly shows that, in the caudal lobules (VII and VIII) of the cerebellum, the lateral edges show deeper invaginations, whereas in the anterior lobe, deeper foliations are found in the medial regions (Fig. 4). Hence, for the anterior lobe, the resulting representation of the cerebellar cortex shows a continuous midline with discontinuous lateral edges, whereas the reverse is found for the more caudal lobules (apart from vermal lobules IX and X). The projections of the vermal and hemispheral parts of lobule VI are more complex and rely in part on a lateral unfolding of Crus 1. Due to the expansion of its hemispheres, the vermal region of lobule VI is also shown discontinuous. The paraflocculus, which at some point is continuous with the ventral part of the copula pyramidalis (lobule VIII), and the adjacent flocculus are unfolded in lateral direction, but the resulting parafloccular/floccular projection, for the sake of figure space, is rotated 90 degrees caudalward. Hence, the flocculus, as the final hemispheral lobule, is pointed in a caudal direction. This conversion was performed manually, and rostrocaudal distances were based on the distance between the plotted sections and were related to the distances in the transverse plane.

Mossy fiber terminals in every folium were counted in 200-μm-wide "bins" of granule cell layer and the resulting numbers were aligned according to the position of the patches within the flattened and outstretched diagram of the cerebellar cortex. No countenance was given to variations in the depth of the granular layer. In areas where it was not possible to divide the patches with parallel lines, the mean width of 200 μm was estimated at the half depth of the granular layer. In this way, a matrix was derived which was visualized with Matlab routines (The MathWorks, Inc.). In a similar way, the regions of molecular layer overlying the patches of granule cell layer that contained one or more labeled climbing fibers were indicated and were also visualized with Matlab. The resulting plots reflect an interpolated, color-coded representation of the density of labeled mossy fiber terminals over the entire

TABLE 1. Numbers of Retrogradely Labeled Neurons Found in Various Precerebellar Centers after CTb Injections into Different Regions of the Vestibulocerebellum[1]

	Medial nodulus						Intermediate nodulus						Flocculus						Ventral paraflocculus		
	832			837			853			852			798			802			836			903		
Brainstem area	Left	Right*	Total	Left	Right*	Total	Left	Right*	Total	Left	Right*	Total	Left	Right*	Total	Left	Right*	Total	Left	Right*	Total	Left	Right*	Total
LRt	6	1	7	4	6	10	2	1	3	3	1	4	35	78	113	16	57	73	18	36	54	6	6	12
Cu/Ecu	0	0	0	3	0	3	0	0	0	0	0	0	0	0	0	1	0	1	0	2	2	0	0	0
Retic. form. medulla	11	10	21	25	29	54	24	30	54	7	3	10	27	34	61	24	21	45	19	24	43	9	17	26
Trigeminal complex	4	0	4	1	0	1	1	2	3	1	4	5	19	36	55	5	13	18	10	20	30	11	14	25
MVe	58	64	122	137	140	277	169	123	292	51	83	134	66	66	132	62	74	136	55	57	112	21	20	41
MVeV	0	0	0	0	0	0	0	0	0	0	0	0	6	0	6	2	2	4	8	10	18	0	0	0
SpVe	1	2	3	12	23	35	16	30	46	5	32	37	20	6	26	20	22	42	25	21	46	30	13	43
SuVe	6	2	8	10	15	25	13	26	39	2	5	7	62	38	100	32	25	57	40	34	74	13	1	14
mlf neurons	4	6	10	15	12	27	10	14	24	4	4	8	13	23	36	22	17	39	43	19	62	3	4	7
PrH	30	31	61	57	63	120	12	15	27	8	8	16	15	20	35	19	21	40	25	18	43	49	44	93
Raphe nuclei			2			7			4			6			9			5			8			3
LC	0	4	4	0	1	1	2	1	3	2	4	6	1	4	5	1	4	5	0	5	5	4	5	9
PB	3	2	5	0	2	2	2	0	2	1	4	5	3	3	6	1	3	4	1	1	2	0	0	0
Retic. form. pons	4	4	8	2	3	5	1	0	1	1	1	2	2	0	2	0	0	0	3	2	5	1	2	3
RtTg	3	1	4	6	1	7	2	6	8	7	5	12	17	9	26	13	3	16	7	3	10	339	157	496
Pn	0	1	1	0	2	2	1	3	4	6	0	6	11	7	18	12	2	14	16	6	22	1724	381	2105
Total	132	128	260	275	301	576	259	251	510	104	154	258	301	329	630	232	267	499	274	262	536	2213	664	2877

[1]Cell counts were made in one of every eight sections.
Asterisk indicates side ipsilateral to the CTb injection. Cu, cuneate nucleus; Ecu, external cuneate nucleus; LC, locus coeruleus; MveV, medial vestibular nucleus, ventral part; PB, parabrachial nuclei; Retic. Form, reticular formation; SpVe, spinal vestibular nucleus; SuVe, superior vestibular nucleus. For other abbreviations, see list.

cerebellar cortex and can be directly compared to the location of the labeled climbing fibers in a similar, but noninterpolated, plot.

Zonal organization of climbing fiber input to nodulus and flocculus

The basic organization of climbing fiber projection to the flocculus and nodulus of the rat has been described by Ruigrok et al. (1992) and Voogd et al. (1996a), respectively (see also Bernard, 1987; Voogd and Ruigrok, 1997)) and will be briefly recaptured here (also see Fig. 9). Due to the angle in the folding of the (para-) floccular lobules with respect to the rest of the cerebellum, the five floccular zones are basically oriented in a transverse direction. From caudal to rostral, these zones are innervated by the rostral-most tip of the medial accessory olive (MAO; C2-zone), VLO (FD-zone), the DC (FE-zone), VLO (FD′-zone), and, finally, again DC (FE′-zone). The FE-zone has a conspicuous continuation into the ventral paraflocculus. However, whereas the parafloccular part is suggested to receive its climbing fibers from the rostral-most part of the DC, the floccular part is innervated from the caudal DC. The VLO zones may be continuous with zones in the ventral paraflocculus that receive their climbing fibers from the caudal-most regions of the ventral leaf of the PO and, therefore, by definition, relate to the cerebellar D-zones (Voogd and Glickstein, 1998). This organizational pattern basically resembles that found in other mammals such as rabbit and cat (Gerrits and Voogd, 1982; Tan et al., 1995), but it is not known if the response patterns of Purkinje cells to optical flow fields (e.g., van der Steen et al., 1994) are also similar. Five rostrocaudally arranged zones have also been identified in nodulus. A medial zone (zone N_{med}) receives its climbing fibers from the DC of the inferior olive and is flanked laterally by an intermediate region (zones N_{int}) that is mainly targeted by the β subnucleus. Centrally within this intermediate region, a thin strip of climbing fibers is labeled from injections into the VLO of the principal olive (PO). Finally, at the lateral border, again a zone is found that receives its climbing fibers from the DC (zone N_{lat}, also see Fig. 9).

RESULTS

General appearance of labeling

All injections resulted in a circular deposit of tracer visualized as a deeply dark-brown or black region without any obvious structure and with a diameter between 0.3 and 0.6 mm (Figs. 1B, 3F). Because at all injection sites Purkinje cell activity had been recorded, the injection sites were usually centered on the molecular or Purkinje cell layer of the nodulus, flocculus, or ventral paraflocculus but also involved the granular layer. The white matter mostly was only marginally involved. Both the DAB-incubated (brown) as well as the DAB/Co^{2+}-incubated sections generated a similar distribution of labeled structures. However, the background was usually less in the DAB material, which therefore was used for descriptive and plotting purposes.

Both retrogradely and anterogradely labeled structures were observed. All injections resulted in retrogradely labeled neurons within the brainstem. Most labeled cells were observed in the vestibular nuclei and inferior olive, in particular within the medial vestibular nucleus (MVe;

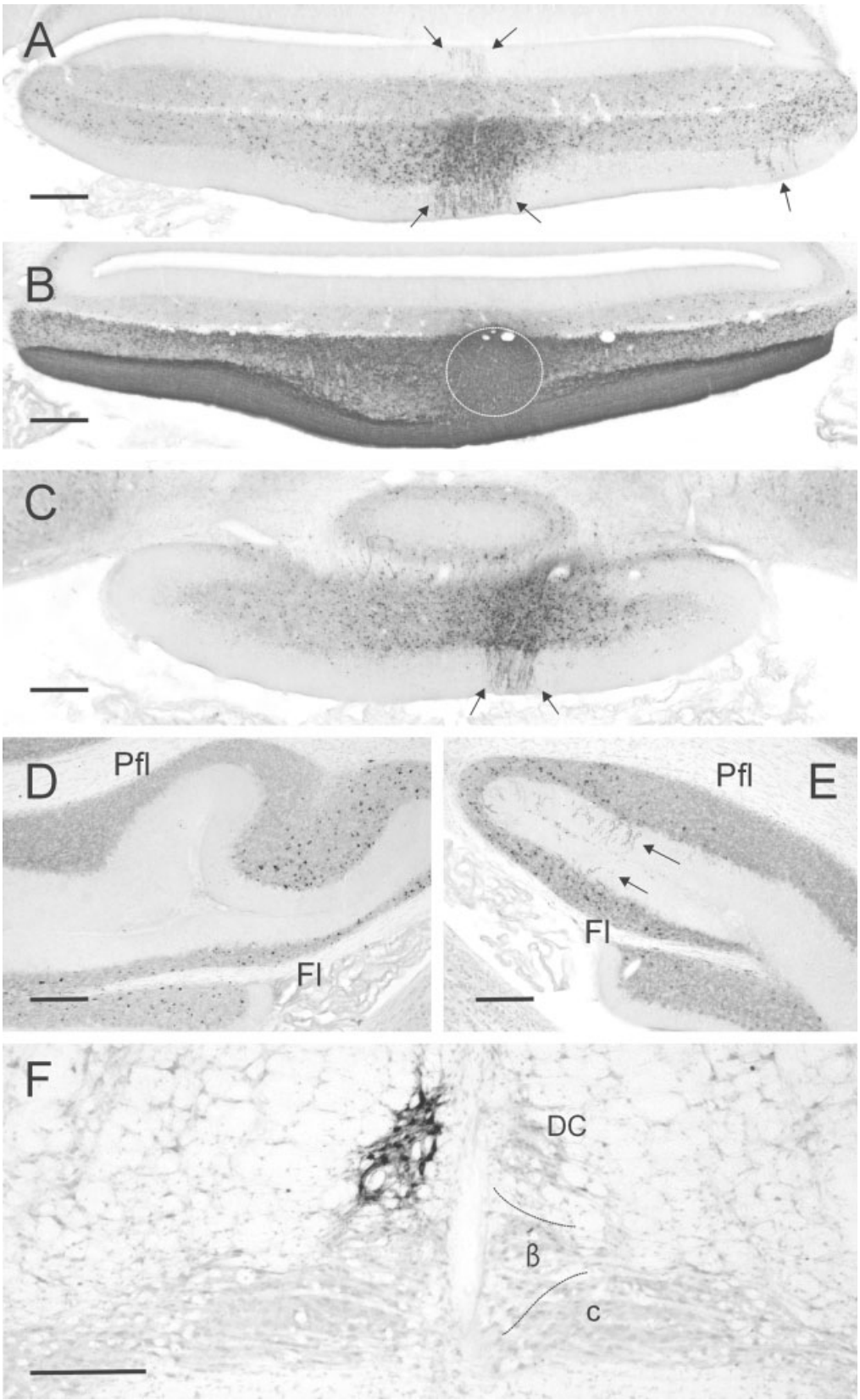

Figure 1

Fig. 3A) and within specific parts of the caudal half of the inferior olivary complex (see below and Fig. 1F). More sparsely, neurons were observed in the lateral reticular nucleus (LRt), scattered throughout the reticular formation and within the reticulotegmental nucleus of the pons (RtTg). An injection in the ventral paraflocculus (903) in addition resulted in huge amounts of labeled neurons in the basal pontine nuclei. Counts of labeled neurons (apart from the inferior olive) are provided in Table 1. Within the cerebellum, nodulus injections resulted in retrogradely labeled neurons in the cerebellar nuclei (mostly the medial cerebellar nucleus) and in unipolar brush cells (UBCs) that were mostly observed within the caudal vermis (Fig. 3C,D). Flocculus injections labeled many neurons within the white matter of the flocculus (Fig. 3E–G) and which may be interpreted as the rat homologue of the basal interstitial nucleus of Langer (Langer, 1985). Both types of injections resulted in huge quantities of granule cells and a corresponding parallel fiber beam that, in case of the nodulus, was usually restricted to the same transverse folium plane in which the CTb injection was placed (Fig. 1B), but in the flocculus ran in a roughly rostrocaudally directed beam (Fig. 3E–G). In addition, anterogradely labeled varicose arborizations were observed in various places of the cerebellum and brainstem. Climbing and mossy fibers that were labeled as the result of axon collateral to collateral transport of CTb (Chen and Aston-Jones, 1998), were usually present near the injection site but also in other parts of the cerebellum. Mossy fiber terminals could be clearly recognized as the classical rosettes usually grouped in patch-like regions within the granular layer (Figs. 1D,E, Fig. 2). Characteristic climbing fiber terminal arborizations were located in the molecular layer and were recognized as two thin parallel lines of varicose fiber fragments. In the flocculus and ventral paraflocculus, a more complete arborization of the individual climbing fiber collaterals was noted, because the orientation of the folia of these lobules is different from the rest of the cerebellum (cf. Figs. 1A,C, 3B,F). Finally, coarse fibers and varicose arborizations were observed in several parts of the cerebellar nuclei and the vestibular nuclei and are mostly thought to be associated with anterogradely labeled Purkinje cell axons and projections. More dispersed fine-caliber varicose arborizations were occasionally noted and are thought to be derived as collaterals of labeled mossy fibers (Voogd et al., 2003).

In the remainder of this study, the distribution of the climbing and mossy fiber collaterals will be further detailed. Because the zonal olivocortical projection is considered to reflect functional entities, the CTb injections will be characterized by their resulting retrograde labeling within the inferior olive. Of the 34 cases, 8 were selected in which the climbing and mossy fiber labeling were plotted and analyzed in detail, thus allowing some concluding remarks on the organization of the mossy and climbing fiber collaterals to the vestibulocerebellum of the rat. The 26 remaining cases were in general concordance with these 8 thoroughly analyzed rats.

Injections into the nodulus

The injections in rats 832 and 837 were both centered at the ventral part of the medial nodulus, the injection in rat 832 being somewhat more restricted compared with that in rat 837. However, in the inferior olive, retrogradely labeled neurons were completely (832) or virtually completely (837) restricted to the DC (Figs. 1, 2, 4), indicating that the injection most likely was confined to the DC-innervated medial strip of the nodulus (zone N_{med}). Mossy fibers in both cases were mostly derived from the parvicellular part of the MVe and the prepositus hypoglossi (PrH; Table 1; Fig. 8). From the plots of Figure 2 (level 9, case 832) and the photomicrographs of Figure 1B (case 837), it can be observed that the granule cell/parallel fiber labeling at the same transverse level of the injection site completely obscured mossy and climbing fiber labeling within the ventral half of the nodulus. However, within the dorsal half of the nodulus, and in the ventral half only several hundreds of microns more rostrally or caudally, climbing and mossy fiber collateral labeling could be clearly recognized (Figs. 1A,C; 2, levels 7 and 11). Note that the mossy fiber rosettes are distributed throughout the width of the nodulus but display a tendency to be clustered within a central and lateral region (Fig. 2, levels 7 and 11). Mossy fiber rosettes, in addition, were found scattered throughout the caudal vermis (lobules VI to IX) and even more sparsely in the vermal and paravermal regions of lobules I and II. A more conspicuous collateralization was observed to reach the flocculus and ventral paraflocculus on both sides of the brain (Figs. 1D,E, 2, levels 13,15). Climbing fiber collaterals were noted directly in rostrocaudal association with the injection site but also in the dorsal half of the nodulus. Climbing fiber labeling in dorsal and ventral nodular parts was continuous in the nodular apex (Fig. 2, level 7). Some isolated climbing fibers were found in the midline vermis of various folia of the uvula (lobule IX, Fig. 2, level 5, also see Fig. 4). A more coherent separate strip-like region of labeling was observed at the lateral edge of the nodulus, most prominently at its ventral half (Figs. 1A, 2, level 11) and was taken to reflect labeling within zone N_{lat}. In addition, climbing fiber terminal labeling was present in a localized region of the ipsilateral flocculus and the ventral paraflocculus surrounding the depth of the posterolateral fissure (Figs. 1E, 2, level 13, 3B). Note that the region of granular layer directly subjacent to the molecular layer containing labeled climbing fibers also displays a high density of mossy fiber rosettes.

Fig. 1. Photomicrographs showing aspects of injection site and resulting labeling in rat 837. **A:** The nodulus shown at a level of 640 μm caudal to the center of the injection site. Note the climbing fiber labeling in the molecular layer (arrows) and the numerous labeled mossy fiber rosettes (and unipolar brush cells) within the granular layer. Also note that a higher density of labeling in the granular layer accompanies the lateral patch of climbing fiber labeling. **B:** The injection site, demarcated by a white circle, is situated medially in the right-hand side of the nodulus and mostly involves the granular layer of the ventral half of the nodulus. Note the relative scarcity of labeling in the dorsal half and the massive parallel fiber labeling in the molecular layer. **C:** Nodular labeling 640 μm rostral to the center of the injection site. Labeled climbing fibers are indicated by arrows. **D,E:** The flocculus (Fl) and ventral paraflocculus (Pfl) at the contra- (D) and ipsilateral (E) side of the cerebellum. Note the labeled mossy fiber rosettes around the depth of the posterolateral fissure on both sides and the labeled climbing fibers (arrows) in E. Also note the correspondence of mossy and climbing fiber labeling in E. **F:** Labeling in the inferior olive was limited to the contralateral dorsal cap. The β-subnucleus and group c of the medial accessory olive are also indicated. Scale bars = 250 μm in A–F.

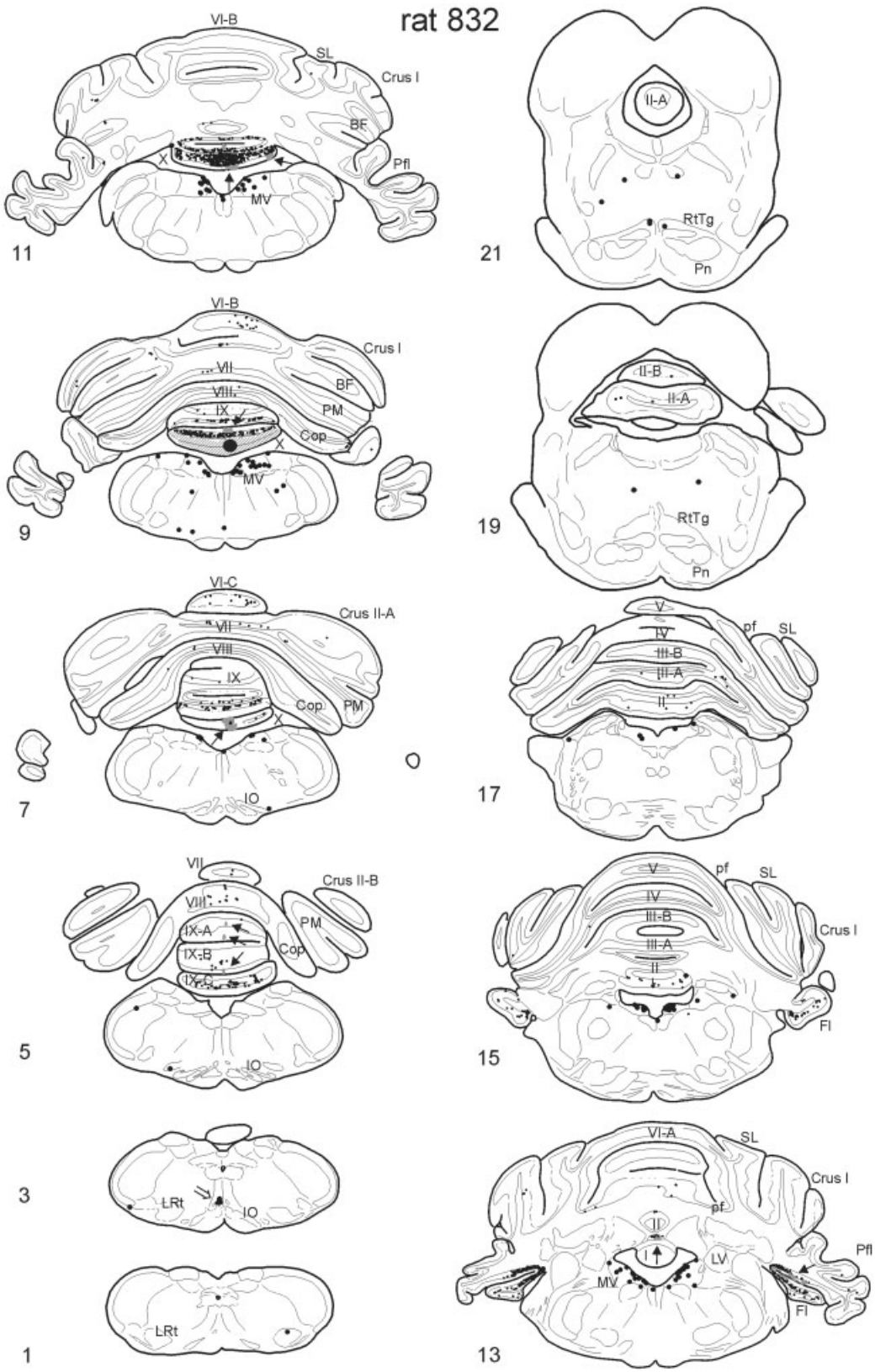

Fig. 2. Diagram depicting cholera toxin b-subunit labeling resulting from the nodulus injection in rat 832. Individual plots are numbered consecutively from caudal (1) to rostral (21) and numbers refer to the consecutive sections of a single vial that consisted of a complete one of eight series of 40-μm sections. Retrogradely labeled neurons in the brainstem (dots: one dot equals one cell), anterogradely labeled mossy fiber rosettes (small dots: one dot equals one rosette), and climbing fiber terminals (gray regions indicated by arrows) were plotted. The injection site is visualized as a black spot in level 9. Hatching indicates a region with massive amounts of labeled granule cells and parallel fibers that obscured all other labeling. An open arrow in level 3 indicates labeling in the dorsal cap of the inferior olive. BF, "buried" folium; Cop, copula pyramidis; Fl, flocculus; IO, inferior olive; LRt, lateral reticular nucleus; LV, lateral vestibular nucleus; MV, medial vestibular nucleus; pf, primary fissure; Pfl, paraflocculus; PM, paramedian lobule; Pn, basal pontine nuclei; RtTg, reticulotegmental nucleus of the pons; SL, simple lobule.

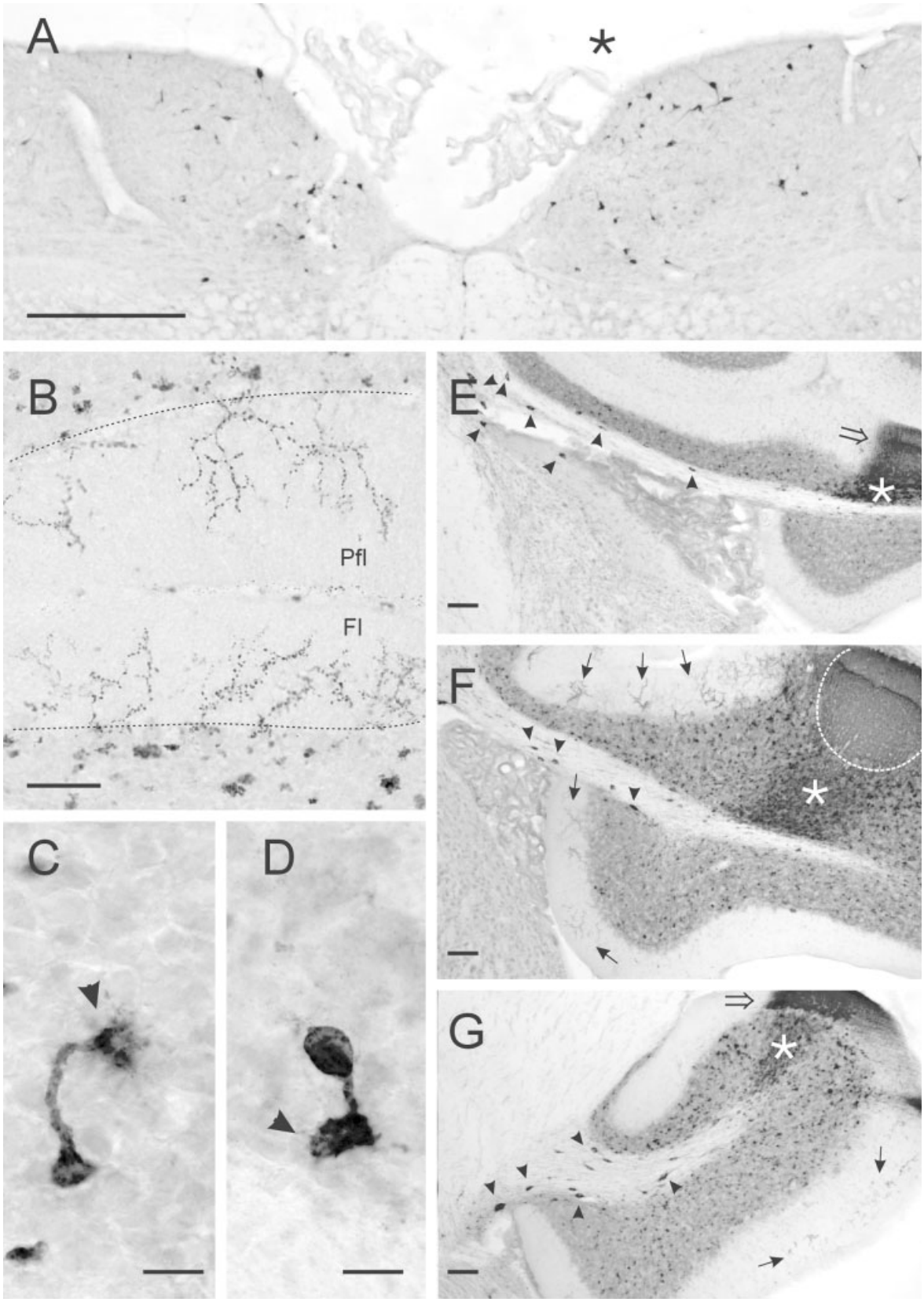

Fig. 3. Photomicrographs depicting aspects of labeling in rats 837 (A–D) and 836 (E–G). **A:** Retrograde labeling of neurons in the medial vestibular nucleus as the result of cholera toxin b-subunit injection in the nodulus (right-hand side, asterisk). Note that the distribution of labeled neurons is similar at both sides of the brain. **B:** Detail of labeling around the depth of the posterolateral fissure showing labeled climbing fibers in the molecular layer and labeled mossy fiber rosettes in the granular layer of the flocculus (Fl) and ventral paraflocculus (Pfl). The dotted line indicates the Purkinje cell layer. **C,D:** Labeled unipolar brush cells in the ventral uvula (C) and nodulus (D). The arrowheads indicate the characteristic brush. **E–G:** Three levels of the flocculus of rat 836. The injection site is indicated by a white circle in F. Note the labeled climbing fibers in the molecular layer (arrows). The parallel fiber bundle (open arrows) emanating from the injection site runs in a roughly rostrocaudal direction and can be seen 480 μm rostral (E) and caudal (G) of the level shown at F. Labeled granule cells located directly subjacent to the parallel fiber bundle are indicated by asterisks. Arrowheads in E, F, and G indicate labeled neurons in the floccular white matter. Scale bars = 500 μm in A; 50 μm in B; 10 μm in C,D; 100 μm in E–G.

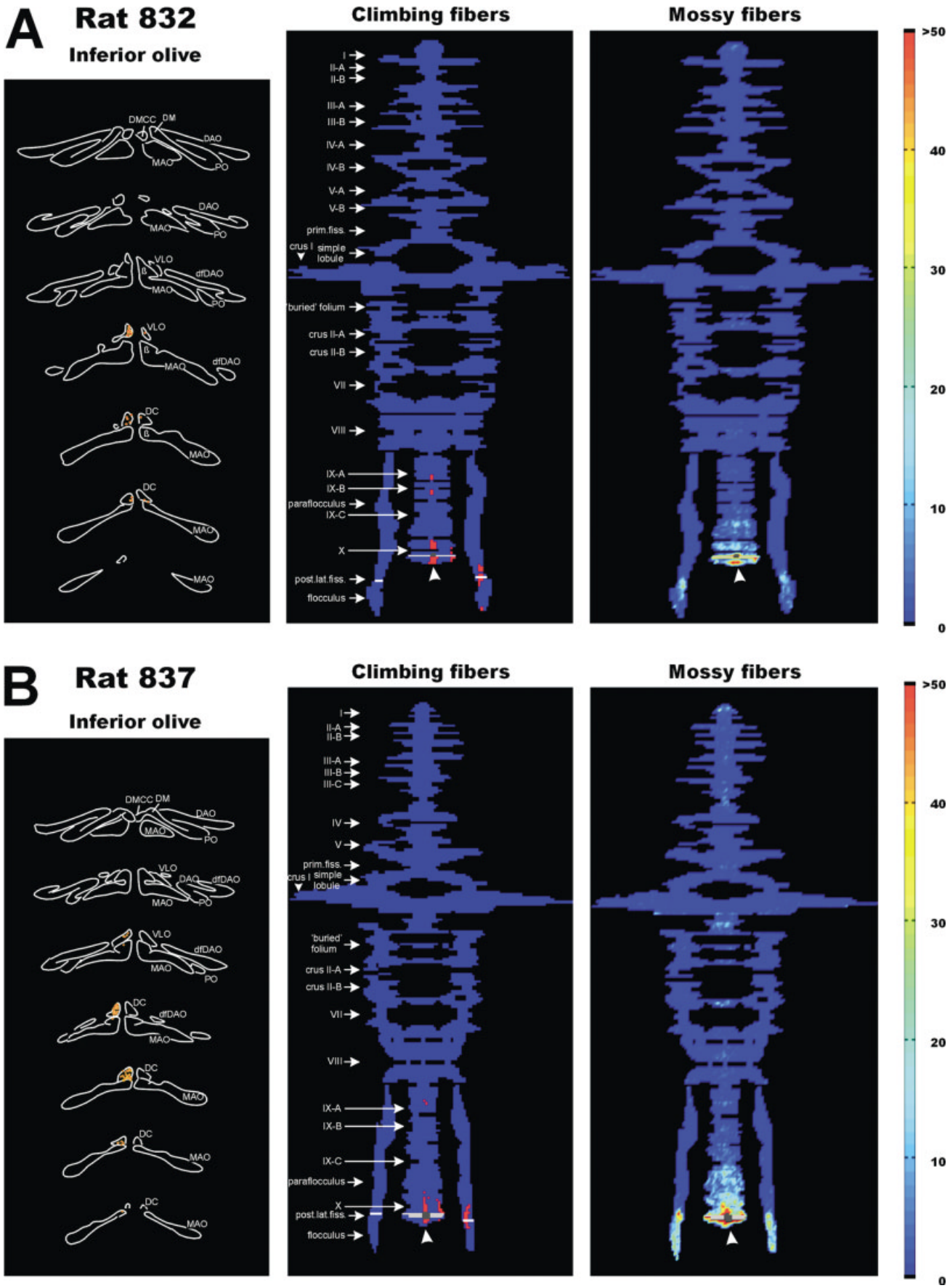
A
Rat 832
Inferior olive
DMCC
DM
DAO
MAO
PO
VLO
dfDAO
DC
Climbing fibers
Mossy fibers
I
II-A
II-B
III-A
III-B
IV-A
IV-B
V-A
V-B
prim.fiss.
crus I simple lobule
'buried' folium
crus II-A
crus II-B
VII
VIII
IX-A
IX-B
paraflocculus
IX-C
X
post.lat.fiss.
flocculus
>50
40
30
20
10
0
B
Rat 837
Inferior olive
DMCC DM
DAO
MAO
PO
VLO
dfDAO
DC
Climbing fibers
Mossy fibers
I
II-A
II-B
III-A
III-B
III-C
IV
V
prim.fiss.
crus I simple lobule
'buried' folium
crus II-A
crus II-B
VII
VIII
IX-A
IX-B
IX-C
paraflocculus
X
post.lat.fiss.
flocculus
>50
40
30
20
10
0

Figure 4

Figure 4 summarizes these results by displaying the number of plotted mossy fiber rosettes in a 200-μm-wide strip of granular layer as a measure of density in a color-coded map of the unfolded and outstretched cerebellar cortex (see Materials and Methods section). In addition, the regions of directly associated molecular layer containing labeled climbing fibers are indicated in a similar map. Finally, plots of retrogradely labeled cells in the caudal part of inferior olive provide information on the cortical zone on which the CTb injection was centered. The diagrams of Figure 4 clearly show that the olivary labeling is restricted to the DC and displays the highest concentration of neurons in its rostral part. In the central panel of the diagram, two strips of climbing fiber collaterals are indicated in the caudal vermis, one next to the midline; the other in the lateral extremity of the lobule. The labeling in flocculus and paraflocculus forms a continuum located around the depth of the posterolateral fissure. It is noteworthy that the highest concentration of mossy fiber rosettes in both cases is also found in the same region, but in addition has a contralateral counterpart as can be clearly seen in the right-hand side of the diagram panels. Mossy fibers are furthermore distributed throughout the nodulus and ventral folium IX-C. It is obvious that both rat 832 (Fig. 4A) and rat 837 (Fig. 4B) essentially show similar results, the main difference being that the injection site in rat 837 was somewhat larger compared to rat 832, which correlated well with a higher density of mossy fiber rosettes. Hence, these two cases attest that this method of analysis was reliable.

Figure 5 shows the summarized results of two injections directed at more lateral aspects of the nodulus. In rat 853 (Fig. 5A) the injection was centered on the dorsal part of the nodulus and resulted in retrograde olivary labeling within the β-subnucleus and of a few cells in the VLO. This injection, therefore, was confined to the intermediate part of the nodulus (N_{int}). A single cell was furthermore observed in the dorsomedial cell column (DMCC). Climbing fiber collaterals were all found in a single zone extending from the ventral nodulus to lobules IX-C and -B and within the depth of lobule IX-A and VIII. No climbing fiber collaterals were found within the flocculus/paraflocculus. Similar to the two previous cases (832 and 837), mossy fibers originated mostly from the MVe but less prominently from the PrH (Table 1; Fig. 8). Labeled mossy fiber rosettes were mainly observed throughout the nodulus and ventral surface of lobule IX-C, however, without any obvious organizational pattern. Scattered terminals were furthermore observed in the remaining parts of lobule IX and within the vermal regions of lobules VIII, IV, III, and II, where they were mostly located in the depth of the lobules. A fair amount of labeled terminals was located in lobule I and, in bilaterally symmetric locations, within the flocculus. The injection in rat 852 (Fig. 5B) was also directed at the intermediate levels of the nodulus but was somewhat smaller and was centered on its ventral surface. Within the olive, labeling was more or less equally distributed within the β-subnucleus and the VLO. Only a single cell was observed in the DC as was one in the contralateral DMCC. Within the caudal vermis, the collateral climbing fiber labeling was virtually identical to that observed in rat 853. However, in addition, three patches of labeled climbing fibers were observed in the hemisphere. In the flocculus, climbing fiber labeling was observed in both the caudal and rostral parts of its dorsal surface and may be taken to reflect labeling in the FD- and FD′-zones. A third, small patch of fibers was located in the lateral-most aspect of crus I. Huge quantities of mossy fibers terminals were observed in the ventral nodulus but were found more scattered to the lateral regions of its dorsal surface, and in lobule IX-C. Patches of labeled rosettes were also found in corresponding regions of the caudal part of the ipsi- and contralateral flocculus, but with some additional patches in more rostral regions.

Fig. 4. Summary diagrams of rats 832 (**A**) and 837 (**B**) with cholera toxin b-subunit injections in the medial part of the nodulus. The left-hand panels show series of plots (interval, 160 μm) of the location of retrogradely labeled neurons in the caudal part of the inferior olive. Caudal is toward the bottom. Every labeled neuron is indicated by a red dot. The middle panels show the unfolded and flattened diagram of cerebellar cortex. The apexes of the respective lobules are indicated by arrows and the posterolateral fissures by white lines. The injection sites are shown as black dots in the ventral (i.e., caudal) part of the nodulus (arrowheads). Gray lines indicate the emanating parallel fiber bundle. Red labeling indicates 200-μm-wide regions of molecular layer that contains one or more labeled climbing fibers. The right-hand panels show the same figurine of the cerebellar cortex but showing the location and, in a color-coded way, the density of labeled mossy fiber rosettes. The bars indicate the number of rosettes counted in a 200-μm-wide bin of granular layer. The figure was devised with Matlab using interpolation. Note that the injections in both rats were centered on the medial part of the ventral nodulus and have similar results with respect to (1) their resultant labeling in the inferior olive (virtually exclusively in the rostral parts of the DC), (2) their climbing fiber collateral labeling (three zones, two of which are found in the medial and lateral nodulus, and one around the depth of the posterolateral fissure), and to (3) their mossy fiber collateral labeling. Also compare the diagram of rat 832 with the plots of Figure 2. See text for further details. Abbreviations used in olive plots: β, β-subnucleus; DAO, dorsal accessory olive; DC, dorsal cap; dfDAO, dorsal fold of DAO; DM, dorsomedial group of PO; DMCC, dorsomedial cell column; MAO, medial accessory olive; PO, principal olive; VLO, ventrolateral outgrowth.

Injections into the flocculus/ventral paraflocculus

Figure 6 shows the results of rats 836 and 802. Here, both injections were centered at the dorsal surface of the flocculus, although the injection in case 836 was located more toward the apex of the lobule (also see Fig. 3F). Retrograde labeling in the inferior olive was found as two distinct patches in the DC and VLO (Fig. 6A), and, therefore, is consistent with the notion that the injection involved at least two floccular zones. Collateral climbing fiber labeling was observed as a more or less continuous zone throughout the rostral-most aspect of the flocculus. Note that the parallel fiber beam emanating from the injection site was positioned perpendicular to the main direction of the climbing fiber zone (also see Fig. 3E–G). A few climbing fibers were labeled at the lateral-most aspect of the ventral nodulus. Mossy fiber rosettes were observed throughout the flocculus but not in the ventral paraflocculus. A very high density of collateral rosettes was also located in the contralateral flocculus. Furthermore, mossy fiber terminals were noted in the caudal vermis (in particular, within ventral lobule IX-C) and throughout vermal, paravermal, and even hemispheral regions of the rest of the cerebellar cortex, with the exception of lobule VIII, and with a rather high density in lobule I. Terminals were mostly observed in the depth of the fissures and showed a symmetrical, patchy pattern at both sides of the brain.

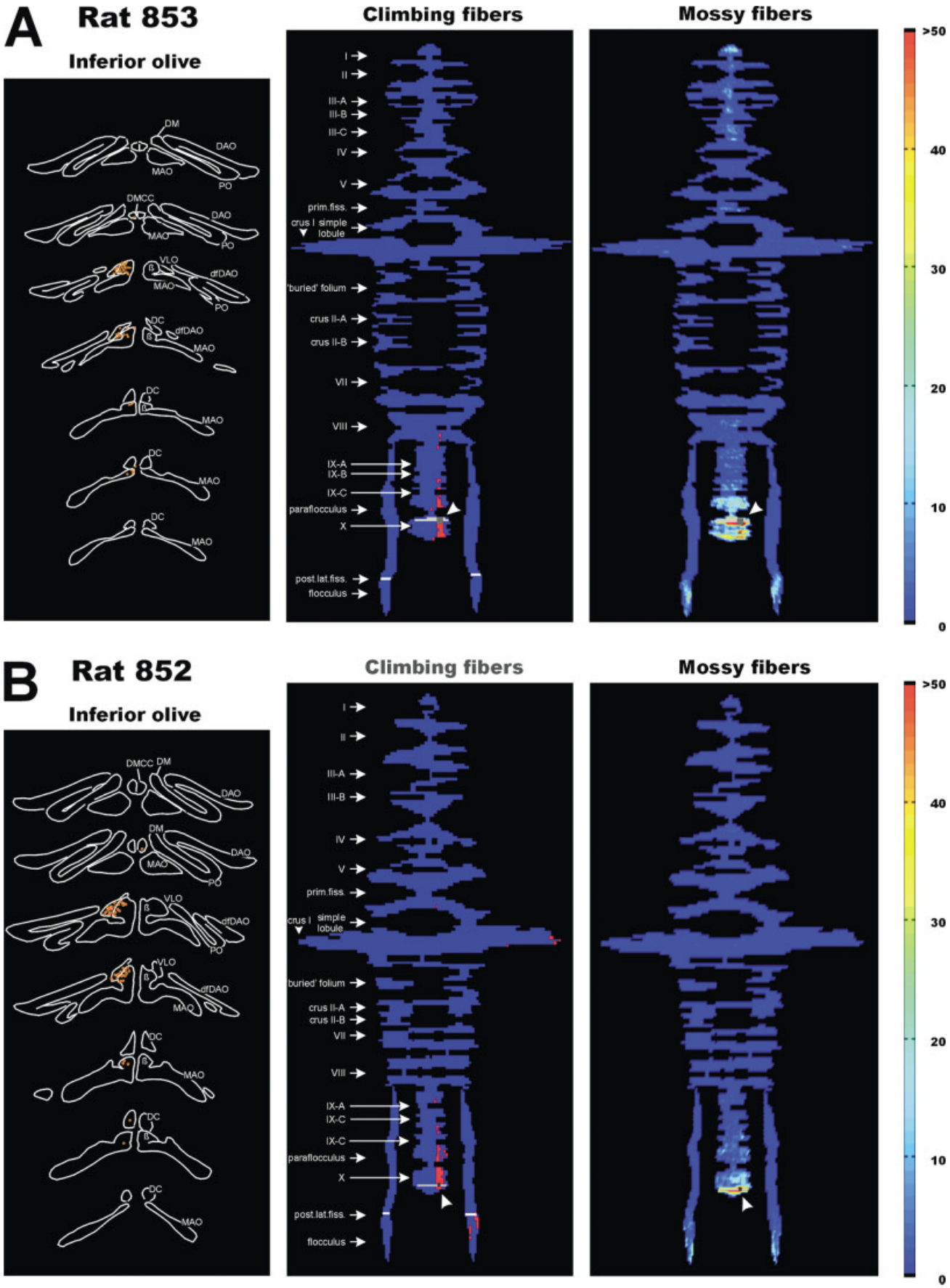

Fig. 5. Summary diagram for rats 853 (**A**) and 852 (**B**) with injections in the intermediate region of the nodulus. Same conventions as Figure 4. See text for further description.

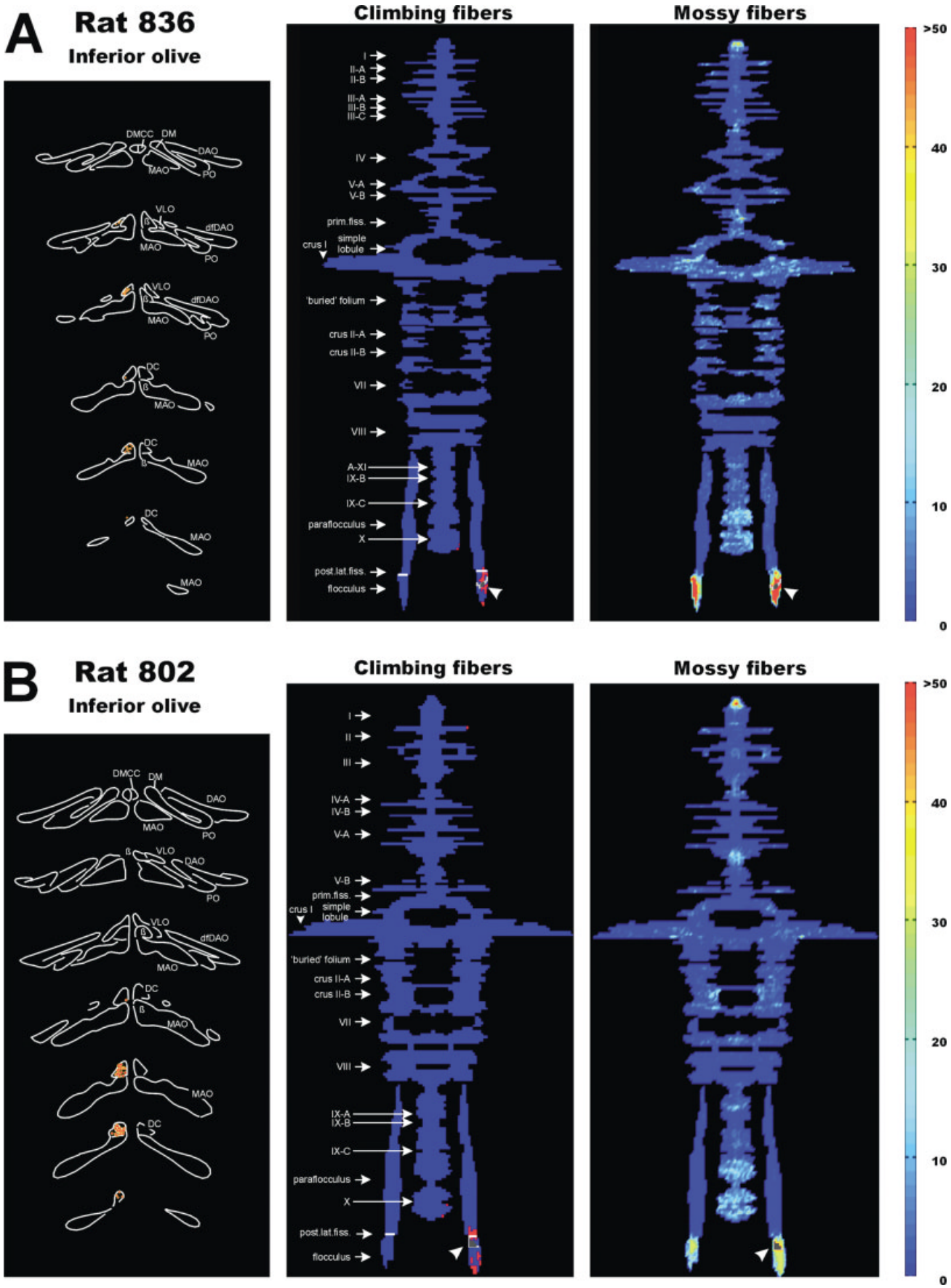

Fig. 6. Summary diagram for rats 802 (**A**) and 836 (**B**) with injections in the flocculus. Same conventions as Figure 4. See text for further description.

The injection in rat 802 (Fig. 6B) resulted in olivary labeling that was confined to the DC, especially to its caudal aspects. This finding would suggest that tracer uptake was restricted to a single climbing fiber zone, which would be the FE-zone (see Fig. 9). Climbing fiber labeling within the flocculus had a somewhat fragmentary character but mostly involved the transition region with the ventral paraflocculus and the dorsal surface of the flocculus. Several labeled climbing fibers were observed at the lateral aspect of the ventral nodulus as well as a few terminal fibers in the lateral part of lobule II. The distribution of labeled mossy fiber rosettes was very similar to that of rat 836, but with less involvement of the hemispheres.

A single floccular VLO zone was specifically injected in rat 798 (Fig. 7A) as was evident from the finding that retrogradely labeled olivary neurons were only found in the VLO. Collateral labeling of climbing fibers was found within a caudally and a more rostrally located floccular band, reflecting the FD- and FD′-zones, respectively (cf. Fig. 9). In addition, a few labeled fibers were found in the intermediate part of the ventral nodulus and at the lateral-most aspect of crus I. Mossy fibers, like those in rats 836 and 802, originated from a wide variety of precerebellar sources and with a less prominent impact from the MVe compared to the nodulus injections (Table 1; Fig. 8). High densities of mossy fiber rosettes were noted throughout both the ipsi- and contralateral flocculus but were not abundant in the caudal vermis. Distinctly higher concentrations were noted in the paravermal and hemispheral regions of crus I and II as well as in the vermal depth of the primary fissure and within lobule I. As in the earlier described cases, a bilaterally symmetrical arrangement was evident throughout the left and right side of the cerebellum.

Finally, rat 903 was chosen for reconstruction (Fig. 7B) since its CTb injection was centered on the ventral aspect of the paraflocculus, close to the bottom of the posterolateral fissure. Labeled olivary cells were observed in the rostral MAO, ventral leaf and bend of the PO and within the rostral-most part of the DC. This distribution implies that the injection site covered the C2-, D-, as well as the parafloccular part of the FE-zone (cf. Fig. 9; Ruigrok et al., 1992). Climbing fiber collaterals were observed in a wide zone in most parts of the paraflocculus and as a narrow strip at the lateral edge of lobule VIII and crus I. In the caudal vermis, two strips of fibers were evident. One was positioned medially (zone N_{med}) and one laterally (zone N_{lat}) within the nodulus. The latter zone appears to be continuous with a similarly placed strip in lobule IX-B/C but which is not reached by olivary neurons of the DC (Voogd and Ruigrok, 1997). As in rat 802, a few fibers were noted laterally within lobule II. Mossy fibers mostly originated from the basal pontine nuclei (Pn) and RtTg. However, apart from these sources a conspicuous input was noted from especially the PrH and other nuclei known to provide input to the vestibulocerebellum (Table 1; Fig. 8). In correspondence with the different precerebellar labeling, the distribution of mossy fiber rosettes was very different from the three cases with injections in the flocculus. High density labeling was found throughout the paraflocculus but only sparsely within the ipsilateral flocculus. In the contralateral flocculus, rosettes were mainly confined to the depth of the posterolateral fissure. Within the ventral nodulus, two conspicuous patches with rosettes were observed at its lateral edges and, more sparsely, within its medial parts. An ipsilateral zone of terminal labeling furthermore was found at the lateral-most aspect of the different folia of lobule IX. The vermis of caudal aspects of lobule VI and of lobule VII contained a high concentration of labeled rosettes (mostly ipsilaterally) as well as the lateral edges of the ipsi- and contralateral crus I. From Figure 7B, it will be evident that the mossy fiber labeling in lobule IX and in crus I matches exactly with the regions of labeled climbing fibers. This aspect of climbing fiber and mossy fiber colocalization was quite specifically illustrated by a very small cluster of labeled mossy fiber terminals that was found in the same position where the small patch of labeled climbing fibers in lobule II was observed (double arrowheads in Fig. 7B).

Conclusions

The eight selected cases confirm earlier descriptions of the organization of the olivary projection to the rat vestibulocerebellum (Ruigrok et al., 1992; Voogd et al., 1996a). However, they also illustrate the arrangement of climbing fiber collateral projections to the vestibulocerebellum as schematically indicated in Figure 9.

The injections in rats 832 and 837 show that olivary neurons in the more rostral parts of the DC supply the medial zone of the nodulus and display a tendency to collateralize to the lateral-most zone of the nodulus as well as to the ventral paraflocculus and adjacent dorsomedial part of the flocculus (FE-zone). This arrangement was verified in rat 903, with an injection centered on the ventral paraflocculus and labeling climbing fibers within both the lateral and medial zones of the nodulus. The intermediate zone of the nodulus is supplied by the β-subnucleus (rat 853) and, in addition, also by the VLO (rat 852). Because only the latter case showed labeling in the flocculus, it can be inferred that only the VLO neurons may collateralize to this lobule, where their climbing fibers are arranged in two strip-like zones. This is in full agreement with the location of the zonal arrangement of these collaterals within the flocculus and with the notion that the β-subnucleus does not provide projections to the flocculus (Ruigrok et al., 1992). The climbing fibers originating from the caudal part of the DC are mostly directed to the flocculus, although it cannot be excluded that some collaterals may also reach the lateral-most zone of the nodulus (rats 802 and 836). The VLO supplies climbing fibers to a rostral (FD′) and a caudally located zone (FD) in the flocculus. Collaterals of at least some of these fibers have been shown to reach the intermediate zone in the nodulus (rats 852 and 798). In addition, at least some VLO neurons supply climbing fibers to the lateral-most edge of crus I (not shown in Fig. 9).

A systematic study on the origin of the mossy fiber rosettes was beyond the scope of the present study; however, based on the cell counts (Table 1; Fig. 8), certain conclusions can be made. The nodulus receives a more prominent input from parvicellular part of the MVe compared with the flocculus. Also, the PrH mostly provides mossy fibers to the medial nodular zone (N_{med}: receiving climbing fibers from the rostral DC) and the ventral paraflocculus (also receiving rostral DC climbing fibers). In rat, the RtTg does not seem to distribute differently to flocculus or nodulus but is rather heavily involved in projections to the ventral paraflocculus. Finally, more sparse projections from the LRt reach the nodulus compared with the

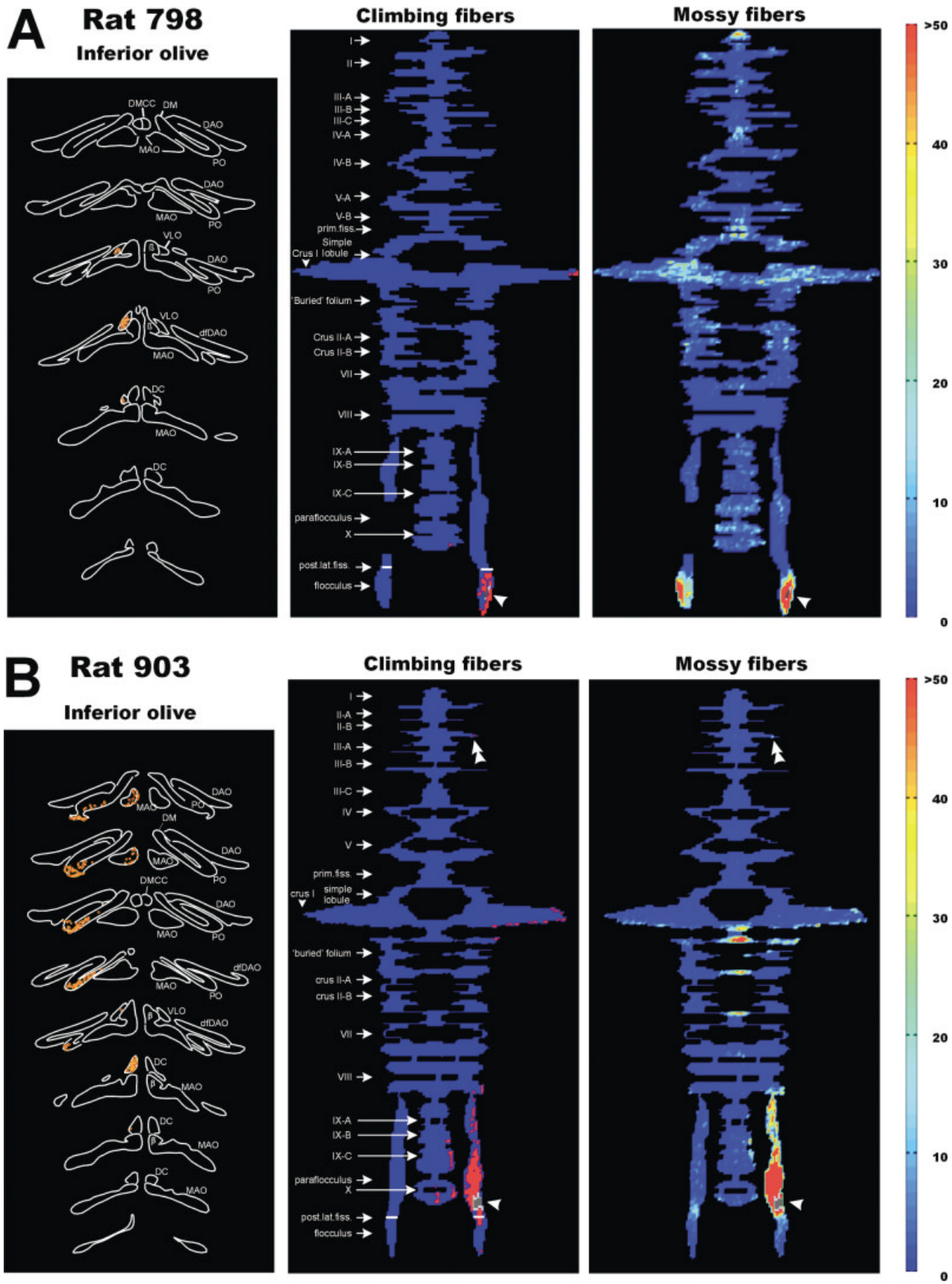

Fig. 7. Summary diagrams for rat 798 (**A**), with an injection in the flocculus, and for rat 903 (**B**), with an injection in the ventral paraflocculus. Same conventions as Figure 4. Double arrowheads in B indicate the position of a few labeled climbing fibers and a related small patch of labeled mossy fibers in the hemisphere of lobule II. See text for further description.

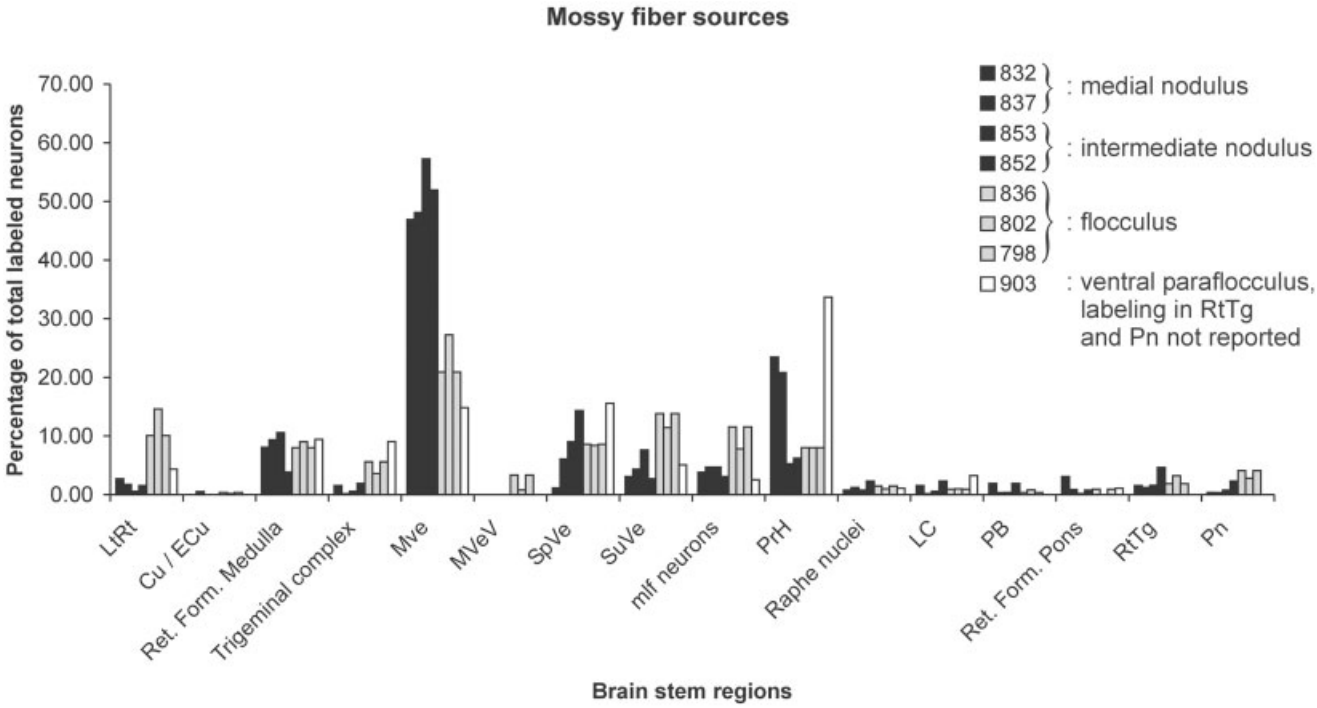

Fig. 8. Bar diagram of the relative distribution (indicated as the percentage of total labeled neurons) of labeled neurons in a number of precerebellar sources of mossy fibers. Bars are arranged from left to right, according to case numbers presented in figure key, from top to bottom. Note that the labeling in RtTg and Pn in rat 903 was not reported to enable a more direct comparison of nuclei providing mossy fibers to the vestibulocerebellum (cf. Table 1). Also note the more widespread distribution of labeled neurons in the floccular cases compared to the nodular cases where most labeled neurons were located in the MVe. See text for further description. Cu, cuneate nucleus; Ecu, external cuneate nucleus; LC, locus coeruleus; LtRt, lateral reticular nucleus; MveV, medial vestibular nucleus, ventral part; PB, parabrachial nuclei; Retic. Form, reticular formation; SpVe, spinal vestibular nucleus; SuVe, superior vestibular nucleus. For other abbreviations, see list.

flocculus. Concerning the distribution of mossy fiber collaterals, it is noted that mossy fibers that terminate in a particular region have a propensity to provide collaterals to other vestibulocerebellar regions in a bilaterally symmetric pattern. This pattern is obviously less well demarcated compared with the climbing fiber zones. Indeed, at best only a tendency of enhanced collateral labeling was observed in the central and lateral aspects of the nodulus (rats 832 and 837). However, nodular derived patches of labeling in the flocculus/ventral paraflocculus were usually quite well demarcated (e.g., rats 832, 852, 853). Another more general conclusion relates to the finding that the nodular injections resulted in mossy fiber collateral labeling that was mostly restricted to the vestibulocerebellum. However, floccular derived mossy fiber collaterals were distributed over more widespread areas of the cerebellar cortex. Finally, and most interesting, it was frequently observed that patches of mossy fiber rosettes were usually found in direct association to the overlying molecular regions that contained collateral climbing fiber labeling. For example, note the distribution of climbing fiber labeling and mossy fiber labeling in the paraflocculus, uvula, crus I, and lobule II of rat 903, and in the flocculus, nodulus, and crus I of rat 798. Similarly, the floccular climbing fiber labeling resulting from nodular injections was clearly related to the patches of mossy fiber collateral labeling (rats 832 and 837).

DISCUSSION

Methodologic considerations

The technique used in the present study has attractive potential for studying collateralization in brain regions (Chen and Aston-Jones, 1998; Voogd et al., 2003). The possibility of collateral to collateral labeling was already described for wheat germ agglutinin–coupled horseradish peroxidase (Mesulam, 1982). However, the labeling by CTb clearly surpasses that of WGA-HRP or other known tracer substances (Chen and Aston-Jones, 1998). Nevertheless, presently, it is not clear if the CTb technique does label all existing collaterals; for example, is a minimum number of uptake points within the injection site necessary to warrant successful labeling of all collaterals? From inspection of the labeled structures, it is clear that, although the density of labeling may vary somewhat, it is usually never a problem to determine whether a particular mossy fiber rosette or climbing fiber is actually labeled or not. The same conclusion can be made on the basis of the labeling characteristics of the retrogradely labeled cells. Vestibular as well as the olivary cells are invariably densely labeled. This finding would suggest that a single mossy fiber rosette or climbing fiber collateral that is located within the injection site might be sufficient to label both the parent neuron as well as its collaterals. It is conspicuous that the label is mostly present within the cell body and dendrites and within their terminal arboriza-

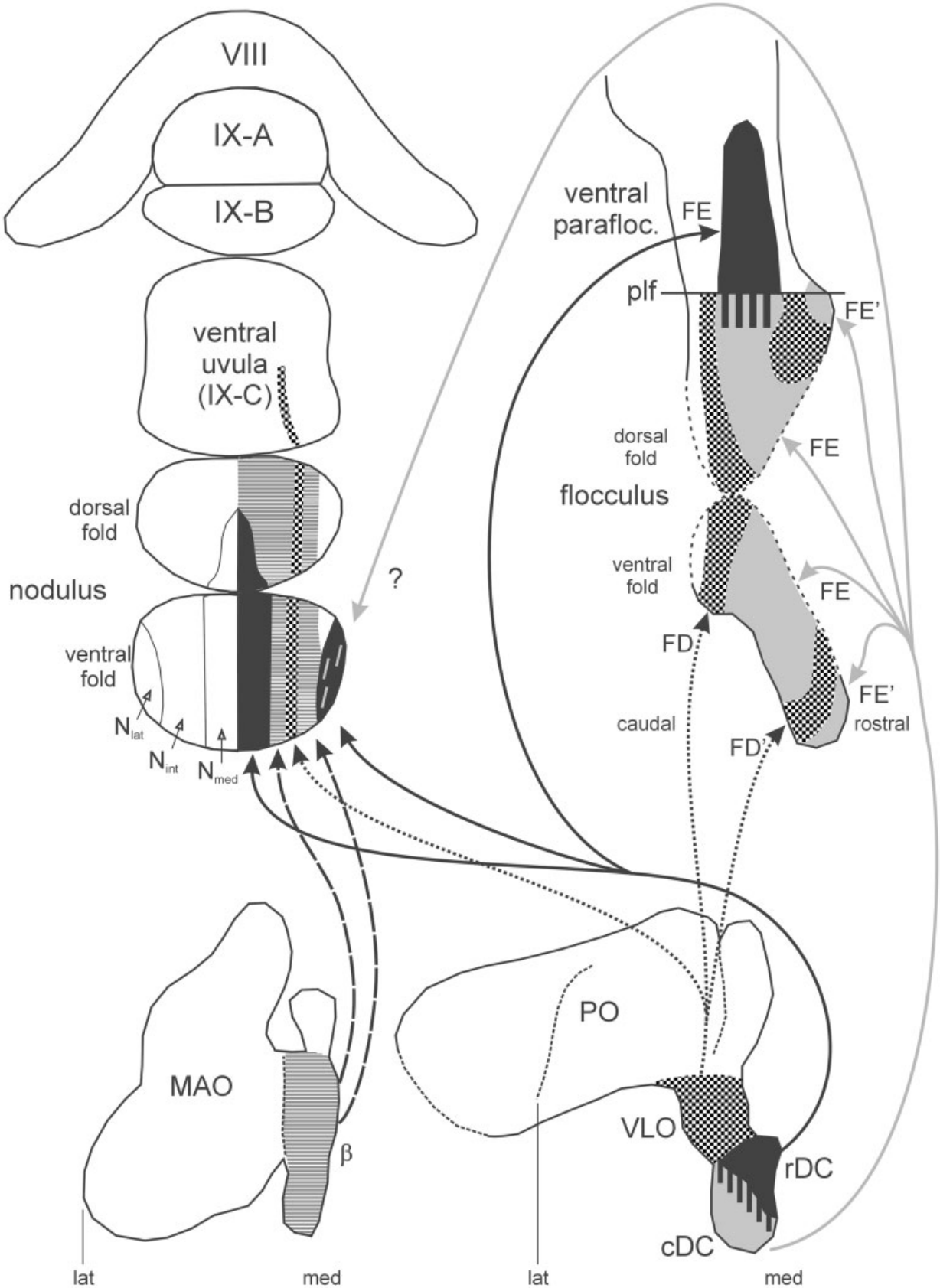

Fig. 9. Diagram summarizing results of climbing fiber collateralization to the vestibulocerebellum. Contours of the left flattened and unfolded medial accessory olive (MAO) and principal olive (PO; Ruigrok and Voogd, 2000) are shown at the bottom. Representations of the unfolded caudal vermis (Voogd et al., 1996a) and right flocculus/ventral paraflocculus (Ruigrok et al., 1992) are shown at the top. Shading and hatching indicate matching regions in the inferior olive and cerebellar cortical zones (N_{med}, N_{int}, and N_{lat} for the nodulus; FD, FE, FD′, and FE′ for flocculus and ventral paraflocculus). Lines indicate collateralization as suggested by the present study. These connections do not suggest that all olivary neurons located in particular areas do indeed collateralize to all related regions of the cerebellar cortex. rDC, rostral dorsal cap; cDC, caudal dorsal cap; β, β-subnucleus; plf, paraflocculus.

tion, but only very poorly in the main axon branches. In case of the mossy fibers, actually only the rosettes were labeled. Thus, it was not possible to follow the routes along which the collaterals between various regions were connected (Voogd et al., 2003).

The technique used in the present study of plotting and visualizing summarized data of mossy and climbing fiber distributions over the entire cerebellar cortex has not been used before and clearly has advantages, but some drawbacks should also be mentioned. It is obvious that the cerebellar cortex is a complex structure, consisting of numerous folded lobules and folia that show indentations of variable depths. Therefore, although the general idea is usually clear, certain subjective judgments are invariably necessary to connect lobules/folia of one section to the next. Furthermore, it will be obvious that the general shape of the resulting diagram is strongly dependent on the angle of sectioning. Both factors will result in some interanimal variability of the resulting diagrams. Finally, and most importantly, to come to the quantification of mossy fiber rosettes, the granular layer was divided in bin-like regions of 200 μm. However, the depth of granular layer was not taken into account. Obviously, this aspect can vary considerably, depending on whether the section was taken at the lobules' top, depth, or oblique to foliations. This indicates that the used color-coding does not reflect actual concentrations of rosette labeling in a sense of number/volume but rather provides an estimate of these densities. However, because these limitations will be similar in all analyzed cases, the diagrams still provide a powerful way of evaluating and comparing the mossy fiber concentrations in such a complex structure as the cerebellar cortex.

Climbing fiber collateralization

The present results are in good agreement with earlier reports showing a distinct zonal arrangement of the climbing fibers to the nodulus and the flocculus and their origin from particular subnuclei of the inferior olive (Bernard, 1987; Ruigrok et al., 1992; Voogd et al., 1996a; Voogd and Ruigrok, 1997; Osanai et al., 1999). However, in addition, the present experiments indicate that the separation of the DC into a rostral and caudal part, as was suggested for the projections to the flocculus/paraflocculus (Ruigrok et al., 1992; Osanai et al., 1999), also can be recognized in the projections to the nodulus. In particular, this study shows that N_{med} does not appear to receive afferents from the caudal-most regions of the DC. Apart from N_{med}, a lateral zone (N_{lat}) innervated by climbing fibers originating from the DC was also recognized by Voogd et al. (1996a), but not by Bernard (1987). In rabbits, the DC has also been divided into a rostral and caudal part. However, here, the anatomical and physiological characteristics of the rostral DC are more in line with the VLO (Leonard et al., 1988: Tan et al., 1995). Also, although a lateral DC-zone has been described in the rabbit the medial-most nodular zone is innervated by the β-subnucleus and is flanked by a zone innervated by the caudal DC. A zone innervated from the rostral DC/VLO lies interspersed between both zones that are supplied by the caudal DC (Tan et al., 1995; Voogd et al., 1996a). As yet, it is not clear to what extent these differences between rat and rabbit (1) are based on definition of regions, (2) are the result of a species difference that reflect a different functional and/or developmental organization, or (3) have a methodologic origin.

The retrograde olivary labeling arising from the floccular/paraflocculus injections also confirmed the layout of the five parallel zones in the flocculus (Ruigrok et al., 1992; Balaban et al., 2000). In rabbit, similarly organized zones have been shown to be involved in the control of eye movement along specific axes (van der Steen et al., 1994). Like the situation in the nodulus, it appears that the definition of caudal DC/rostral DC/VLO is different for both species (Ruigrok et al., 1992). Because no similar studies have been published for the rat flocculus, it is presently not known to what extent the identified rat floccular zones reflect the same functional organization as shown in the rabbit.

Electrophysiological and retrograde double-labeling studies already indicated that individual olivary cells can send collaterals to both the nodulus and flocculus (Takeda and Maekawa, 1989a,b). Here, we confirm this notion for the rat and include an organizational pattern of this collateralization (Fig. 9). Indeed, olivary neurons in the caudal-most part of the DC do not seem to reach the nodulus, whereas the more rostrally located DC cells collateralize to N_{med} and N_{lat} and/or to the continuation of the FE-zone into the ventral paraflocculus. Olivary neurons in the VLO may collateralize to nodulus as well as to the VLO zones in the flocculus. In addition, this study provides additional evidence that some collaterals may also reach the lateral edge of crus I.

Obviously, the present study only allows conclusions on the occurrence of collateralization between certain zones and does not provide an indication on the actual number of neurons that do provide collaterals to different zones. Also, it is not possible to indicate whether or not single neurons can provide more than two zones with climbing fibers, e.g., the present technique cannot distinguish between the situation where the collaterals of a subgroup of neurons in the rostral DC provides the lateral nodular zone with climbing fibers while another subgroup provides the ventral paraflocculus/dorsal surface flocculus with collaterals with the situation where all three regions can be supplied by the same neuron. The observation that both in rat 837 as well as in rat 903, the rostral DC does not appear to contain a major population of unlabeled neurons would argue in favor of the last option. However, this particular issue can only be positively resolved with single anterograde or multiple retrograde tracing studies. In the rabbit, Takeda and colleagues provided electrophysiological and double-labeling data that suggest that, at best, about a third of the DC neurons might reach both nodulus and flocculus. For the VLO, that number would be approximately half of that of the DC (Takeda and Maekawa, 1989a,b). These data indicate that, although collateralization of the climbing fibers to separate but functionally related regions does occur, the actual numbers of olivary neurons doing so may be limited. Nevertheless, the present material suggests that climbing fiber collateralization between zones may be quite robust in specified regions.

Mossy fiber collateralization

The present study confirms that most of the precerebellar mossy fiber sources to the vestibulocerebellum are found in the vestibular nuclei and the PrH (Voogd et al., 1996a,b). Moreover, the nodulus/ventral uvula is innervated by primary vestibular fibers. A primary vestibular innervation of the flocculus is either absent or restricted to

particular subdivisions of the flocculus/ventral paraflocculus (Blanks et al., 1983; Gerrits et al., 1989; Osanai et al., 1999). Additional sources for the flocculus that were verified in the present study include the LRt and neurons located within and around the medial longitudinal fascicle (mlf), including the paramedian tracts (Yamamoto, 1979; Blanks et al., 1983; Gerrits et al., 1984; Akaogi et al., 1994; Buttner-Ennever and Horn, 1996; Osanai et al., 1999). In contrast to earlier studies (Blanks et al., 1983), the RtTg and Pn was only found to act as a prominent source of mossy fibers when the ventral paraflocculus was incorporated in the injection site (rat 903). In rabbit and primates, the secondary vestibulocerebellar fibers (Thunnissen et al., 1989) and the mossy fibers derived from the nucleus PrH (Belknap and McCrea, 1988) and the adjoining reticular formation (Buttner-Ennever and Horn, 1996) are distributed in bilaterally symmetric patterns, which are likely to result from intracerebellar decussations (Wu et al., 1999). CTb injections into the nodulus invariably result in a mostly focal, injection site-dependent distribution of labeled rosettes in the ipsi- and contralateral flocculus. Moreover, in some experiments, there was some evidence of a zonal distribution in the nodulus as well. Also, sparse but usually patchy concentrations of labeled rosettes are seen in depth of the vermis as can be seen in case 837, where these patches are neatly aligned in the rostrocaudal direction with the injection site. Also in cases with injections in the flocculus, it was obvious that collaterals frequently collateralize to the nodulus and to the contralateral flocculus. However, contrary to the nodulus injections, labeled mossy fiber terminals in addition were observed over wide regions of the cerebellar cortex, including the hemispheres. Invariably, the bilaterally symmetrical character of the labeling was maintained in these cases. Because the flocculus, compared with the nodulus, receives a more prominent input from the LRt, mlf and paramedian tracts, trigeminal complex, superior vestibular nucleus, and Pn, it can be hypothesized that specifically these neurons are likely candidates to supply mossy fibers to the flocculus as well as to many other nonvestibular regions of the cerebellum. A major projection to the flocculus arising from the RtTg, raphe nuclei, and dorsomedial reticular formation (Blanks et al., 1983; Gerrits and Voogd, 1986; Akaogi et al., 1994) that could underlie the observed differences in nodular versus floccular collateralization, could not be substantiated in the present study. Alternatively, the difference in collateral involvement may arise from different collateralization characteristics of particular populations of vestibulocerebellar or prepositus hypoglossal neurons, e.g., in rabbit, it has been shown that vestibular neurons that project to flocculus may have a somewhat different distribution compared to those that innervate the nodulus (Epema et al., 1990), although both populations consist of approximately the same numbers and are found at both sides of the vestibular nuclei.

The only exception with respect to the bilateral symmetry of the mossy fiber collateralization was noted in case 903, where the injection was centered on the ventral paraflocculus and where the mossy fibers were mainly distributed ipsilaterally over virtually the whole paraflocculus, but contralaterally only to the part of the ventral paraflocculus that was directly adjacent to the flocculus. This finding suggests that only the mossy fibers related to the DC-innervated part of the ventral paraflocculus send their collaterals to the homologous part on the contralateral side, whereas the surrounding regions of the ventral paraflocculus are innervated by the pontine nuclei (Osanai et al., 1999) and collateralize only ipsilaterally. Note that this may also relate to the region of the lateral crus I that also contains labeled climbing fibers and where in the same region high densities of labeled mossy fiber rosettes were observed on both sides of the brain. The conspicuous collateralization of mossy fibers to the vermis of lobules VI and VII was virtually absent from nodular or floccular CTb injections and suggests collateralization of mossy fibers between the ventral paraflocculus and saccade-related regions of the cerebellar vermis (Noda and Fujikado, 1987; Godschalk et al., 1994).

An aspect that may clearly obscure the pattern of collateralization of mossy fibers is found in the in- and output characteristics of the UBCs, which have been shown to be particularly prevalent in the uvulo-nodular lobe (Berthie and Axelrad, 1994; Mugnaini and Floris, 1994; Dino et al., 1999, 2000; Nunzi and Mugnaini, 2000). These cells have been shown to terminate with intralobular mossy fiber-like rosettes. Because many of these neurons were recognized as retrogradely labeled cells in the nodulus and ventral uvula after the nodular injections, their axonal terminations, as well as the terminals of the UBC somata that were located within the injection site cannot be distinguished from the mossy fiber rosettes originating in the precerebellar centers. A way to solve this problem would be to double label these intrinsically originating mossy fiber rosettes with immunohistochemical techniques against UBC-specific antigens such as calretinin and metabotropic glutamate receptor mGluR1 (Nunzi et al., 2002). It may be expected that in this situation, the organizational pattern of the vestibulocerebellar mossy fibers to the nodulus may become more differentiated and, in addition, could provide a means to study potential patterning of UBC organization.

Relation between climbing and mossy fiber patterns

One of the most intriguing findings was the virtual constant relation between regions with climbing fiber collaterals and a patch of labeled mossy fiber rosettes located directly subjacent to them. Clear examples are found in cases 837 and 903, in the latter case even a few, apparently "lost" climbing fibers were accompanied by a small patch of mossy fiber rosettes. Obviously, mossy fiber collaterals can be distributed over more widespread areas but are usually found in the same region as the climbing fiber collaterals as well. The same conclusion was reached in a recent study investigating mossy and climbing fiber collateralization reaching the paramedian lobule and copula pyramidis (Voogd et al., 2003). Hallem et al. (1999) have noted a relation between the tactile fields in the granular layer and the overlying pattern of zebrin labeling of Purkinje cells. Because the zebrin pattern is closely related to the pattern of climbing fiber projection (Gravel et al., 1987; Voogd et al., 1996a; Voogd and Ruigrok, 1997; Voogd et al., 2003), these observations suggest that there may be a clear spatial relationship between the receptive fields of the mossy fibers and organization of climbing fibers. Indeed, several physiological studies have indicated that the receptive field of the mossy fibers is similar to that of the climbing fibers in the same cerebellar cortical focus (Eccles et al., 1968; Ekerot and Larson, 1980;

Brown and Bower, 2001). Mossy fibers induce Purkinje cell simple spike firing by means of the granule cell–parallel fiber connection, whereas the climbing fibers induce complex spike triggering of the Purkinje cells, thus enabling a discrimination of both types of afferent activation. Despite the diverging nature of the parallel fiber, a remarkable and rather consistent finding relates to the observation that the simple spike receptive field in many instances is very similar to that of the subjacently positioned mossy fibers. This has been attributed to the dominant effect of the ascending segment of the granule cell axon (Llinas, 1982; Gundappa-Sulur et al., 1999). The observation that the receptive fields of climbing fiber responses and mossy fiber–parallel fiber responses seem to be quite similar has led Bower and colleagues to suggest that the classic Marr–Albus hypothesis that proposes that the climbing fiber signal relays an error/training signal that will alter the Purkinje cell's simple spike response to parallel fiber input, may need serious reconsideration (Brown and Bower, 2001). They argue that most of the hypotheses on cerebellar learning, and specifically those on associative learning, require that different types of signals are transmitted by means of the mossy and climbing fiber pathways. This would seem to contradict a systematic conjunctional activation of mossy and climbing fibers. However, in our study, although we note a clear correspondence between climbing and mossy fiber collateralization, it is obvious that the mossy fiber collaterals cover wider cerebellar regions compared to the climbing fibers. This finding would indicate that within a particular focus with climbing fibers, also many unlabeled mossy fibers must still be present with an unknown origin and, on the other hand, labeled mossy fibers are found in regions with unlabeled climbing fibers, which consequently may have a quite different receptive field than that of the labeled mossy fibers. Also, a recent study in decerebrate cats indicates that the simple spike and mossy fiber receptive fields may be quite different (Ekerot and Jorntell, 2001) and, as far as the simple spike receptive field is concerned, rather variable (Jorntell and Ekerot, 2002), suggesting that the relation between the mossy fiber/parallel fiber-evoked simple spikes and the climbing fiber-evoked complex spikes may not be so straightforward.

Functional considerations

The DC and VLO are known to relay signals from the accessory optic system to the flocculus (Leonard et al., 1988) and are likely to be involved in adaptive control of vestibulo- and optokinetic ocular reflexes (Nagao et al., 1997), whereas the β-subnucleus relays vestibular signals and is suggested to be involved in postural reflexes (Barmack et al., 1993). Here, we show that DC and VLO neurons may connect to multiple regions in either flocculus or nodulus. Indeed, one single homogeneous region of the inferior olive such as the VLO may reach as many as four separate regions in the cerebellar cortex, i.e., an intermediate strip in the nodulus, two strips in the flocculus, and the lateral edge of crus I. Although the present technique does not provide information on quantitative aspects of actual collateralization, it is likely that these regions all process a similar type of olivary information. Moreover, there is reason to assume that at least part of the mossy fiber input to the same regions is also similar. Presently, it is not known if, and if so how, the cerebellum makes a functional use of these various regions. Studies in the rabbit using white matter stimulation of identified floccular zones has provided some evidence that different zones with the same climbing fiber input result in the same type of induced eye movements (van der Steen et al., 1994). However, Apps and Lee (1999) recently showed that cerebellar regions that belong to the same zone and that receive climbing fiber input from the same part of the inferior olive (and to a limited extent also by means of collateralizing branches; Apps, 2000)) may behave quite differently during a behavioral task. Therefore, at this point, it is far from clear what the rationale for the existence of multiple climbing fiber zones would be. It will be necessary to investigate in detail to what extent these collateralizations are typical in other cerebellar areas (Voogd et al., 2003) and how they relate to the mossy fiber collateralization. Most importantly, however, detailed information is required if and how these various distributed cerebellar regions react to physiologically relevant stimuli (e.g., Apps and Lee, 1999; Hallem et al., 1999; Brown and Bower, 2001; Ekerot and Jorntell, 2001).

ACKNOWLEDGMENTS

The author thanks Erika Sabel-Goedknegt for expert histologic assistance. Hans van der Burg and Eddie Dalm provided technical and photographic assistance. Prof. dr. Jan Voogd provided helpful comments on the article.

LITERATURE CITED

Akaogi K, Sato Y, Ikarashi K, Kawasaki T. 1994. Mossy fiber projections from the brain stem to the nodulus in the cat. An experimental study comparing the nodulus, the uvula and the flocculus. Brain Res 638:12–20.

Apps R. 2000. Rostrocaudal branching within the climbing fibre projection to forelimb- receiving areas of the cerebellar cortical C1 zone. J Comp Neurol 419:193–204.

Apps R, Garwicz M. 2000. Precise matching of olivo-cortical divergence and cortico-nuclear convergence between somatotopically corresponding areas in the medial C1 and medial C3 zones of the paravermal cerebellum. Eur J Neurosci 12:205–214.

Apps R, Lee S. 1999. Gating of transmission in climbing fibre paths to cerebellar cortical C1 and C3 zones in the rostral paramedian lobule during locomotion in the cat. J Physiol 516:875–883.

Apps R, Trott JR, Dietrichs E. 1991. A study of branching in the projection from the inferior olive to the x and lateral c1 zones of the cat cerebellum using a combined electrophysiological and retrograde fluorescent double labeling technique. Exp Brain Res 87:141–152.

Balaban CD, Schuerger RJ, Porter JD. 2000. Zonal organization of flocculovestibular connections in rats. Neuroscience 99:669–682.

Barmack NH, Fagerson M, Fredette BJ, Mugnaini E, Shojaku H. 1993. Activity of neurons in the beta nucleus of the inferior olive of the rabbit evoked by natural vestibular stimulation. Exp Brain Res 94:203–215.

Barmack NH, Errico P, Ferraresi A, Fushiki H, Pettorossi VE, Yakhnitsa V. 2002. Cerebellar nodulectomy impairs spatial memory of vestibular and optokinetic stimulation in rabbits. J Neurophysiol 87:962–975.

Belknap DB, McCrea RA. 1988. Anatomical connections of the prepositus and abducens nuclei in the squirrel monkey. J Comp Neurol 268:13–28.

Bernard J-F. 1987. Topographical organization of olivocerebellar and corticonuclear connections in the rat: an WGA-HRP study. I. Lobules IX, X and the flocculus. J Comp Neurol 263:241–258.

Berthie B, Axelrad H. 1994. Granular layer collaterals of the unipolar brush cell axon display rosette-like excrescences. A Golgi study in the rat cerebellar cortex. Neurosci Lett 167:161–165.

Blanks RH, Precht W, Torigoe Y. 1983. Afferent projections to the cerebellar flocculus in the pigmented rat demonstrated by retrograde transport of horseradish peroxidase. Exp Brain Res 52:293–306.

Brown IE, Bower JM. 2001. Congruence of mossy fiber and climbing fiber tactile projections in the lateral hemispheres of the rat cerebellum. J Comp Neurol 429:59–70.

Buttner-Ennever JA, Horn AK. 1996. Pathways from cell groups of the paramedian tracts to the floccular region. Ann N Y Acad Sci 781:532–540.

Chen S, Aston-Jones G. 1998. Axonal collateral-collateral transport of tract tracers in brain neurons: false anterograde labelling and useful tool. Neuroscience 82:1151–1163.

De Zeeuw CI, Wylie DR, DiGiorgi PR, Simpson JI. 1994. Projections of individual Purkinje cells of identified zones in the flocculus to the vestibular and cerebellar nuclei in the rabbit. J Comp Neurol 349:428–447.

De Zeeuw CI, Hansel C, Bian F, Koekkoek SKE, van Alphen A, Linden DJ, Oberdick J. 1998. Expression of a protein kinase C inhibitor in Purkinje cells blocks cerebellar long term potentiation and adaptation of the vestibulo-ocular reflex. Neuron 20:495–508.

Dino MR, Willard FH, Mugnaini E. 1999. Distribution of unipolar brush cells and other calretinin immunoreactive components in the mammalian cerebellar cortex. J Neurocytol 28:99–123.

Dino MR, Schuerger RJ, Liu Y, Slater NT, Mugnaini E. 2000. Unipolar brush cell: a potential feedforward excitatory interneuron of the cerebellum. Neuroscience 98:625–636.

Eccles JC, Provini L, Strata P, Taborikova H. 1968. Analysis of electrical potentials evoked in the cerebellar anterior lobe by stimulation of hindlimb and forelimb nerves. Exp Brain Res 6:171–194.

Ekerot CF, Jorntell H. 2001. Parallel fibre receptive fields of Purkinje cells and interneurons are climbing fibre-specific. Eur J Neurosci 13:1303–1310.

Ekerot C-F, Larson B. 1980. Termination in overlapping sagittal zones in cerebellar anterior lobe of mossy and climbing fiber paths activated from dorsal funiculus. Exp Brain Res 38:163–172.

Ekerot C-F, Larson B. 1982. Branching of olivary axons to innervate pairs of sagittal zones in the cerebellar anterior lobe of the cat. Exp Brain Res 48:185–198.

Epema AH, Gerrits NM, Voogd J. 1990. Secondary vestibulocerebellar projections to the flocculus and uvulo-nodular lobule of the rabbit: a study using HRP and double fluorescent tracer techniques. Exp Brain Res 80:72–82.

Errico P, Ferraresi AA, Barmack NH, Pettorossi VE. 1996. Role of cerebellar uvula-nodulus in the control of head orientation-specific eye velocity in the rabbit. Ann N Y Acad Sci 781:614–618.

Gerrits NM, Voogd J. 1982. The climbing fiber projection to the flocculus and adjacent paraflocculus in the cat. Neuroscience 7:2971–2991.

Gerrits NM, Voogd J. 1986. The nucleus reticularis tegmenti pontis and the adjacent rostral paramedian reticular formation: differential projections to the cerebellum and the caudal brain stem. Exp Brain Res 62:29–45.

Gerrits NM, Epema AH, Voogd J. 1984. The mossy fiber projection of the nucleus reticularis tegmenti pontis to the flocculus and adjacent ventral paraflocculus in the cat. Neuroscience 11:627–644.

Gerrits NM, Voogd J, Magras N. 1985. Vestibular afferents of the inferior olive and the vestibulo-olivo-cerebellar climbing fiber pathway to the flocculus in the cat. Brain Res 332:325–336.

Gerrits NM, Epema AH, van Linge A, Dalm E. 1989. The primary vestibulocerebellar projection in the rabbit: absence of primary afferents in the flocculus. Neurosci Lett 105:27–33.

Godschalk M, Van der Burg J, Van Duin B, De Zeeuw CI. 1994. Topography of saccadic eye movements evoked by microstimulation in rabbit cerebellar vermis. J Physiol 480:147–153.

Gravel C, Eisenman LM, Sasseville R, Hawkes R. 1987. Parasagittal organization of the rat cerebellar cortex: direct correlation between antigenic Purkinje cell bands revealed by mabQ113 and the organization of the olivocerebellar projection. J Comp Neurol 265:294–310.

Groenewegen HJ, Voogd J, Freedman SL. 1979. The parasagittal zonation within the olivocerebellar projection. II. Climbing fiber distribution in the intermediate and hemispheric parts of cat cerebellum. J Comp Neurol 183:551–602.

Gundappa-Sulur G, De Schutter E, Bower JM. 1999. Ascending granule cell axon: an important component of cerebellar cortical circuitry. J Comp Neurol 408:580–596.

Hallem JS, Thompson JH, Gundappa-Sulur G, Hawkes R, Bjaalie JG, Bower JM. 1999. Spatial correspondence between tactile projection patterns and the distribution of the antigenic Purkinje cell markers anti-zebrin I and anti-zebrin II in the cerebellar folium crus IIA of the rat. Neuroscience 93:1083–1094.

Ito M. 1984. The cerebellum and neural control. New York: Raven Press.

Jorntell H, Ekerot CF. 2002. Reciprocal bidirectional plasticity of parallel fiber receptive fields in cerebellar Purkinje cells and their afferent interneurons. Neuron 34:797–806.

Langer TP. 1985. Basal interstitial nucleus of the cerebellum: cerebellar nucleus related to the flocculus. J Comp Neurol 235:38–47.

Leonard CS, Simpson JI, Graf W. 1988. Spatial organization of visual messages of the rabbit's cerebellar flocculus. I. Typology of inferior olive neurons of the dorsal cap of Kooy. J Neurophysiol 60:2073–2090.

Llinas R. 1982. Radial connectivity in the cerebellar cortex: a novel view regarding the functional organization of the molecular layer. Exp Brain Res Suppl 6:189.

Mesulam M-M. 1982. Principles of horseradish peroxidase neurochemistry and their application for tracing neural pathways. In: Mesulam M-M, editor. Tracing neural connections with horseradish peroxidase. Chichester: John Wiley. p 1–151.

Mugnaini E, Floris A. 1994. The unipolar brush cell: a neglected neuron of the mammalian cerebellar cortex. J Comp Neurol 339:174–180.

Nagao S, Kitamura T, Nakamura N, Hiramatsu T, Yamada J. 1997. Differences of the primate flocculus and ventral paraflocculus in the mossy and climbing fiber input organization. J Comp Neurol 382:480–498.

Noda H, Fujikado T. 1987. Topography of the oculomotor area of the cerebellar vermis in macaques as determined by microstimulation. J Neurophysiol 58:359–378.

Nunzi MG, Mugnaini E. 2000. Unipolar brush cell axons form a large system of intrinsic mossy fibers in the postnatal vestibulocerebellum. J Comp Neurol 422:55–65.

Nunzi MG, Shigemoto R, Mugnaini E. 2002. Differential expression of calretinin and metabotropic glutamate receptor mGluR1alpha defines subsets of unipolar brush cells in mouse cerebellum. J Comp Neurol 451:189–199.

Osanai R, Nagao S, Kitamura T, Kawabata I, Yamada J. 1999. Differences in mossy and climbing afferent sources between flocculus and ventral and dorsal paraflocculus in the rat. Exp Brain Res 124:248–264.

Paxinos G, Watson C. 1986. The rat brain in stereotaxic coordinates. 2nd ed. Sydney: Academic Press.

Payne JN. 1987. Cerebellar afferents from the lateral reticular nucleus in the rat. Neuroscience 23:211–221.

Payne JN, Wharton SM, Lawes INC. 1985. Quantitative analysis of the topographical organization of olivocerebellar projections in the rat. Neuroscience 15:403–415.

Ruigrok TJH, Cella F. 1995. Precerebellar nuclei and red nucleus. In: Paxinos G, editor. The rat nervous system. Sydney: Academic Press. p 277–308.

Ruigrok TJ, Voogd J. 2000. Organization of projections from the inferior olive to the cerebellar nuclei in the rat. J Comp Neurol 426:209–228.

Ruigrok TJH, Osse R-J, Voogd J. 1992. Organization of inferior olivary projections to the flocculus and ventral paraflocculus of the rat cerebellum. J Comp Neurol 316:129–150.

Serapide MF, Panto MR, Parenti R, Zappala A, Cicirata F. 2001. Multiple zonal projections of the basilar pontine nuclei to the cerebellar cortex of the rat. J Comp Neurol 430:471–484.

Sugihara I, Wu HS, Shinoda Y. 2001. The entire trajectories of single olivocerebellar axons in the cerebellar cortex and their contribution to cerebellar compartmentalization. J Neurosci 21:7715–7723.

Sultan F. 2001. Distribution of mossy fibre rosettes in the cerebellum of cat and mice: evidence for a parasagittal organization at the single fibre level. Eur J Neurosci 13:2123–2130.

Takeda T, Maekawa K. 1989a. Olivary branching projections to the flocculus, nodulus and uvula in the rabbit. I. An electrophysiological study. Exp Brain Res 74:47–62.

Takeda T, Maekawa K. 1989b. Olivary branching projections to the flocculus, nodulus and uvula in the rabbit. II. Retrograde double labeling study with fluorescent dyes. Exp Brain Res 76:323–332.

Tan J, Gerrits NM, Nanhoe JI, Simpson JI, Voogd J. 1995. Zonal organization of the climbing fiber projection to the flocculus and nodulus of the rabbit: a combined axonal tracing and acetylcholinesterase histochemical study. J Comp Neurol 356:23–50.

Thunnissen IE, Epema AH, Gerrits NM. 1989. Secondary vestibulocerebellar mossy fiber projection to the caudal vermis in the rabbit. J Comp Neurol 290:262–277.

van der Steen J, Simpson JI, Tan J. 1991. Representation of three-dimensional eye movements in the cerebellar flocculus of the rabbit. In: Schmid R, Zambarbieri D, editors. Oculomotor control and cognitive processes. North Holland: Elsevier. p 63–77.

van der Steen J, Simpson JI, Tan J. 1994. Functional and anatomic organization of three-dimensional eye movements in rabbit cerebellar flocculus. J Neurophysiol 72:31–46.

Voogd J. 1995. Cerebellum. In: Paxinos G, editor. The rat nervous system. Sydney: Academic Press. p 309–350.

Voogd J, Bigaré F. 1980. Topographical distribution of olivary and cortico nuclear fibers in the cerebellum: a review. In: Courville J, de Montigny C, Lamarre Y, editors. The inferior olivary nucleus. Anatomy and physiology. New York: Raven Press. p 207–234.

Voogd J, Glickstein M. 1998. The anatomy of the cerebellum. Trends Neurosci 2:305–371.

Voogd J, Ruigrok TJH. 1997. Transverse and longitudinal patterns in the mammalian cerebellum. In: De Zeeuw CI, Strata P, Voogd J, editors. The cerebellum: from structure to control. Amsterdam: Elsevier. p 21–37.

Voogd J, Gerrits NM, Ruigrok TJH. 1996a. Organization of the vestibulocerebellum. Ann N Y Acad Sci 781:553–579.

Voogd J, Jaarsma D, Marani E. 1996b. The cerebellum: chemoarchitecture and anatomy. In: Swanson LW, Björklund A, Hökfelt T, editors. Handbook of chemical neuroanatomy. Amsterdam: Elsevier. p 1–369.

Voogd J, Pardoe J, Ruigrok TJH, Apps R. 2003. The distribution of climbing and mossy fibre collateral branches from the copula pyramidis and the paramedian lobule. J Neurosci 23:4645–4656.

Wu HS, Sugihara I, Shinoda Y. 1999. Projection patterns of single mossy fibers originating from the lateral reticular nucleus in the rat cerebellar cortex and nuclei. J Comp Neurol 411:97–118.

Wylie DR, De Zeeuw CI, DiGiorgi PL, Simpson JI. 1994. Projections of individual Purkinje cells of identified zones in the ventral nodulus to the vestibular and cerebellar nuclei in the rabbit. J Comp Neurol 349:448–463.

Yamamoto M. 1979. Topographical representation in rabbit cerebellar flocculus for various afferent inputs from the brainstem investigated by means of retrograde axonal transport of horseradish peroxidase. Neurosci Lett 12:29–34.